THE CUTANEOUS TRICHOPHYTIN REACTION.[1]

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PLATE XXXIX.

Until recently the trichophyton and allied infections have been regarded as strictly localized diseases without causing any general changes in the organism. The investigations of Calderone (1), Truffi (2), Plato (3), Pautrier and Lutembacher (4) have shown that in cases of trichophytia profunda the infection leads to changes in the organism aside from the local skin lesions. Patients suffering with trichophytia profunda respond to subcutaneous injections of culture filtrates of different strains of trichophyton, called trichophytin, with local and general reaction. The local reaction occurring on the point of injection gives rise to inflammation and infiltration. Besides this reaction on the point of injection, but less constantly and less pronounced, there occurs another local reaction on the diseased area. The general reaction consists of malaise, headache, muscular pains, rise of temperature, etc. Superficial cases of trichophytia do not give any reaction under the usual conditions. The analogy of this reaction with the tuberculin reaction in cases of tuberculosis is very striking and, indeed, it goes even further, as will be shown later. The trichophytin reaction is just as specific as the tuberculin reaction in the sense that it is not observed in healthy individuals or in patients suffering with diseases not due to an infection with hyphomycetes belonging to the trichophyton group. It is of interest that trichophytins prepared from different types of trichophyton derived from clinically different cases act alike, so that this reaction cannot be utilized for the differentiation of various trichophyton infections. This result is the less surprising, as the investigations of Král (5) and Waelsch

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(6), showing a rather extensive pleomorphism of pathogenic hyphomycetes, taught us to be cautious in basing the classification of these fungi on morphological and cultural differences.

Several investigators tried to produce specific lesions in animals by intravenous subcutaneous and intraperitoneal injections of trichophyton cultures, but without success. Nevertheless, it seems that such injections may produce a certain change in the reactivity of the animals. For instance, Plato injected a trichophyton culture subcutaneously in a rabbit and this animal reacted to an injection of trichophytin given four days later with a definite rise of temperature. The experiments of Citron (7) conducted with the view of producing an active or passive immunity by treating rabbits with trichophyton cultures gave practically negative results. Only a moderate formation of precipitins was found, and again the serum of these rabbits did not only react with the trichophyton used for the immunization, but also with other types.

The investigations of Bloch (8) and Bloch and Massini (9) contributed greatly to the advancement of our knowledge with regard to the altered reactivity of an organism having passed through a trichophyton infection. In his animal experiments he was able to show that a guinea pig having passed through an infection with the culture of a certain trichophyton became immune to a subsequent infection. The unsuccessful attempts of previous investigators to produce immunity by means of intravenous, intraperitoneal and subcutaneous injection prompted him to adopt a different method. The cultures were directly applied to the skin and after a definite period of incubation the disease developed, reached its acme at a definite time and healed spontaneously. The course of the disease is typical and shows a definite division in different periods. A second inoculation given after the spontaneous restitution did not lead to any definite lesions, and it was shown that the immunity was not only limited to that part of the skin which had been the seat of the previous infection, but the whole skin proved refractory to a second inoculation. The intraperitoneal injection of the same culture which on cutaneous inoculation produced immunity was, at the most, capable of diminishing the intensity of a renewed cutaneous infection. It was, furthermore, not possible to obtain an active immuni-

zation of animals by means of "presssaft" or culture filtrates, nor was it possible to immunize animals passively with the serum of immunized animals. But with repeated cutaneous inoculations the time at which the immunity appears could be determined. At intervals of two days inoculations were made around a primary infection. The second and third inoculations, made on the second and fourth days after the primary, were still positive, the fourth, made six days after the primary, led only to reddening and infiltration, while from the seventh day on the inoculations were negative. The time from which the inoculations became negative coincided with the beginning of the spontaneous involution of the first inoculation. The processes, therefore, leading to the spontaneous involution are the same which are responsible for the immunity. These experiments show an unmistakable analogy to those of v. Pirquet with vaccinia. The analogy would be still more striking if it could be shown that the subsequent inoculations in the experiments of Bloch just cited were really not entirely negative, but gave rise to an "early reaction." From this analogy it may be predicted that a repetition of these experiments in man will supply this last link, particularly since Bloch and Massini observed in man a typical "accelerated reaction" in one case. The earlier experiments with trichophytin established an analogy with the reaction observed in tuberculosis, and the latter experiments of Bloch and Massini established a close analogy with the train of events seen in vaccinia, and these authors recognized that the trichophyton infections give rise to an altered reactivity of the organism, the allergy of v. Pirquet. Following this idea, Bloch used the trichophytin for the cutaneous test and with success. Just as v. Pirquet showed that the cutaneous tuberculin reaction was positive in individuals suffering with tuberculosis or having passed through a tuberculous infection, patients suffering with a trichophytia of not too superficial a character gave a positive cutaneous test with trichophytin which could be elicited even long after the infection had disappeared.

Thanks to the kindness of Dr. Bloch in supplying us with his trichophytin, we were able to make a number of observations which will now be described.

Trichophytins.—Plato prepared his trichophytin by growing the fungi in a 3 per cent. maltose bouillon for two and three months at room temperature. The luxuriant growth is ground up as much as possible with a glass rod or strong platinum wire and filtered through a sterile filter-paper. Transfers from the filtrate to agar-agar proved the absence of living fungi. After an addition of 0.25 per cent. carbolic acid, the trichophytin is ready for use. Truffi prepared his trichophytins in a similar manner, but in order to insure their absolute sterility, he filtered through Chamberland candles and boiled the filtrates, or even heated them to 120° in the autoclave without destroying their activity. Furthermore, he was able to extract the active principle from a culture grown on solid media with absolute alcohol. The residue of the evaporated alcoholic extract was dissolved in water and proved active. Ether did not destroy its activity. Pautrier and Lutembacher took a culture grown for fifteen days in solid media, ground it up with serum and heated it at 110°.

Bloch and Massini filtered cultures grown in maltose bouillon through Chamberland candles and preserved the trichophytin by addition of 0.5–1 per cent. carbol-glycerin. The culture must be at least four weeks old. The activity is not diminished by keeping it two to three hours in the thermostat at 37°, and for a longer period of time in the refrigerator. Evaporation *in vacuo* at 40° does not interfere with its activity, but heating under pressure destroys it.

A primary toxicity of the trichophytins seems very doubtful. An experiment of Bloch may be mentioned with regard to the primary toxic effect of trichophytin. A bouillon culture of eight months standing was filtered through a Chamberland filter and the filtrate injected repeatedly, in quantities of 1–2 centimeters, intraperitoneally and subcutaneously in guinea pigs. Within three weeks the weight of the animals had dropped from 600 to 400 grams. He deduced from these experiments a toxic effect. But the condition of the experiments hardly justifies the assumption of a direct toxic action of the trichophytin, which is the substance responsible for the reactions. The vaccinations were made with the undiluted trichophytin, according to the method of v. Pirquet.

Bloch and Massini describe the reaction as follows: At the earliest after four hours, and usually after twelve to twenty-four hours, the reaction appears in the form of a flat red papule of the size of a lentil or pea, causing violent itching. In the following twenty-four hours the papule increases somewhat in size and recedes slowly in the course of the next days; but sometimes it remains for a longer time. Frequently a brownish pigmented spot is seen in its place for several weeks. This is the typical positive reaction. Sometimes and particularly in superficial cases, the papule is smaller and only a little elevated, that is, the reaction is weaker. Healthy individuals never give a positive reaction. The time at which the cutaneous trichophytin reaction occurs was determined by inoculating an individual (Dr. M.) with a small amount of a culture, and from the second day daily cutaneous vaccinations were made with trichophytin obtained from the same culture. The inoculation produced a typical skin lesion. The cutaneous reaction remained negative up to the sixth day. On the seventh day the vaccination elicited a small reaction. The vaccination of the eighth day produced a distinct papule. The vaccination of the following days led to a typical reaction which did not increase in intensity at the subsequent vaccinations. The reaction remains positive long after the disease has run its course, and in one case it occurred two and one-half years after the infection was cured. The intensity of the reaction was proportional to the intensity of the still existing or previously existing disease. The deeply infiltrating forms give the most intense reactions. The reaction was negative in cases where the infection limited itself to the hairs or the superficial horny layers of the epidermis. Cases with some infiltration give a moderate reaction.

Our own experiments were limited to cutaneous vaccinations with trichophytin. Unfortunately we did not have cases at our disposal where a trichophytosis still existed.

The total number of cases vaccinated with trichophytin was 131. The age varied from two weeks to seventy-nine years. The vaccinations were made according to the method of v. Pirquet, and two vaccination points were made with the trichophytin and one control.

The following table records the cases in which a history of

trichophyton infection was obtained and where the reaction was positive.

TABLE I.

History of Trichophytosis and Positive Reaction.

	Age.	History.	Remarks.
1. P.H.	61 years.	29 years ago ringworm on neck, lasting 9 months.	Seen every second day. On the sixth day reaction negative. On the eighth day definitely positive reaction. Papule of 5 mm. diameter, red, elevated. Two days later practically faded, hardly any redness, no elevation.
2. T. B.	67 years.	Ringworm on face and arms, several attacks; the last, 40 years ago.	On second day reaction faintly positive, little raised red papule. Two days later entirely faded.
3. T. D.	63 years.	13 years ago doubtful sycosis.	On second day one point, red, raised papule 6.5 mm. in diameter, the other point faintly, positive. On fourth day first point about same diameter, the other point red, raised papule of 5.5 mm. diameter, On sixth day about the same. Three days later nearly entirely disappeared.
4. Mr. T.	24 years.	When 6 years old, ringworm on face and chin; easily cured, no deeper lesions.	After 6 hours positive reaction, reaching its maximum after 24 hours with a diameter of 7 mm. The size, redness and elevation gradually decreasing within the next 6 days.
5. Dr. B.	29 years.	17 years ago ringworm on face, lasting more than one month. 7 years ago ringworm on genitals, reaching down the thigh half way to knee. Duration 3 to 4 months.	After 24 hours red, raised and slightly painful papule of about 5.5 mm. diameter.
6. N. H.	7 years.	Superficial ringworm on head; has been under treatment.	Microscopic examination did not show any mycelia in hair. After 24 hours red papule slightly elevated, and redness not very intense. Not seen any more.

7. S.	30 years.	When about 6 years old eruption on skin of face. Several other children had the same eruption. Lasted about one week.	After 21 hours red, raised papule increasing in size and intensity during the next 24 hours, gradually disappearing during the next week. Second vaccination positive also.
8. Dr. As.	36 years.	Dhobie itch in Philippines. Last attack 7 months ago. (No other infection with trichophytosis.)	First vaccination positive, very doubtful. Second vaccination 17 days later. After 24 hours red, raised papule about 5 mm. in diameter. 24 hours later diminished much in size and intensity, rapidly disappearing 24 hours later like control.
9. A.	49 years.	Deaf mute. No history obtainable.	After 24 hours slight reaction, red and a little raised. 2 days later about the same. 3 days later nearly like control.

Of the 131 cases, eighty-two were inmates of the tuberculosis ward of the Bay View hospital,[2] and these were observed at two day intervals for at least one week. Seven cases were patients in the Johns Hopkins Hospital, Ward F, and could be observed daily for a sufficient length of time. Ten cases were taken from the children's ward of the Hebrew Hospital. Seventeen cases are represented by students and physicians working in the children's department of the Johns Hopkins University, and fifteen cases were patients in the same department. In these latter cases, the observation could not be continued as long as we might have wished, for frequently the patients did not return more than once or twice. A number of vaccinations could not be recorded at all because the patients either did not return, or returned after too great an interval. Of the 131 cases, 107 were vaccinated simultaneously with tuberculin, bouillon treated exactly like the tuberculin but without any bacteria, and trichophytin. The bouillon vaccinations were made in order to see whether it might give a reaction by itself. In one case only, a doubtful reaction occurred, and this was at the point where the bouillon was inoculated. All the other cases showed a completely negative result.

[2] I wish to acknowledge my indebtedness to Dr. L. F. Barker and Dr. J. Wilson for their permission granting me access to the wards of the Johns Hopkins Hospital and Bay View Hospital.

Twenty-four hours after the vaccination, the measurements were as follows: tuberculin, 4:4 mm., 5:4 mm.; bouillon, 4:4, 4.5:5 (redder and a little more raised than tuberculin and trichophytin); trichophytin, 4.5:3.5, 4:4; control, 3:2.5 mm. Twenty-four hours later: tuberculin, 3:4, 4:3; bouillon, 4:4.5, 5:4; trichophytin, 3:3, 4:4; control, 2.5:2.5. The bouillon vaccination points showed the most pronounced changes, being somewhat more red and elevated than the others, although the reaction was not a very definite one. Twenty-four hours later all the vaccination points were alike. This reaction is given in full, because it was the only one where any doubt was possible with regard to a positive reaction elicited by the bouillon alone.

Besides these 107 cases, ten were vaccinated with tuberculin and trichophytin, one with bouillon and trichophytin, and thirteen only with trichophytin.

It may be remarked that the trichophytin is much darker than the bouillon or the tuberculin, and frequently a pigmentation was seen around the points of vaccination, sometimes arranged in ring form, disappearing in a few days. This pigmentation differs from the pigmentation which is left on the place of vaccination after the disappearance of a positive reaction, and it is due no doubt to the pigment contained in the trichophytin. The tuberculin vaccinations did not give any results not noted before.

Of interest are the two cases giving a history of Dhobie itch (Table I, No. 8; Table III, No. 4). Both were physicians who had spent some time in the Philippines. The first vaccination was negative in both cases; in one a second vaccination could be given seventeen days after the first. Now the reaction, although slight, was definitely positive. The Dhobie itch is, according to Manson (10), a disease not of uniform etiology, but in a certain number of cases hyphomycetes belonging to the trichophyton group seem to play a rôle. The cutaneous test with trichophytin may be of value in distinguishing this group from others due to different infections.

In any case it would be desirable to extend the investigations in this direction. In the one case giving a positive reaction on the second vaccination, it is very probable that here we have an induced reaction like those seen in tubercular individuals where the amount of antibody is insufficient, but where the vaccination stimulates the production of antibodies, so that after a first negative result, the subsequent vaccinations are positive. One other negative result was of special interest. A colored child, eleven months of age, came to

the dispensary with an affection of the skin on the forehead and around the eyes. The center of this affection had cleared, while it was spreading with an infiltrated, slightly inflamed margin. The disease had lasted for some time, withstanding treatment by different ointments. The trichophytin reaction was negative and microscopically no hyphomycetes could be detected in some material taken from the margin. The lesions disappeared entirely under antisyphilitic treatment. There were no other signs of syphilis to be found. Another case deserves special mention. A man 61 years of age gave a history of having had a ringworm 29 years ago. The vaccination points with trichophytin did not show any sign of reaction on the sixth day; on the eighth day there was a definite although slight reaction (Table I, No. 1) which had disappeared two days later. This reaction is analogous with the accelerated reaction seen in cases of vaccination in vaccinia.[3] Similar late reactions like this are seen, although rarely, in tuberculin vaccinations. The explanation in both cases is that the organism has an insufficient amount of antibody at its disposal to react immediately with the vaccine or the tuberculin. The vaccination leads to a renewed formation of antibody which reacts with the vaccine or tuberculin or trichophytin left on the place of vaccination, producing the toxic substance giving rise to the reaction. In this way the slight, rapidly disappearing reaction of our case could be explained, since it is very unlikely that after the lapse of several days much unresorbed trichophytin should have remained on the place of vaccination. In the case of tuberculin such late reactions are extremely rare, and it requires a second vaccination to show this secondary reaction, because none of the tuberculin seems to be left at the place of vaccination. For the same reason, it is not probable that such a late reaction will be seen frequently in the case of trichophytin. In vaccinia the accelerated reaction on relatively late revaccination (corresponding to this type of reaction just described in trichophytin and tuberculin) is a rather regular occurrence and for a very simple reason. Here the allergen consists of living

[3] See v. Pirquet, Fig. 23 in "Klinische Studien ueber Vakzination, etc.," Leipzig, 1907. In this figure there occurs a misprint. The lines recording the events in first vaccinations and revaccinations are interchanged.

organisms which even multiply. Whenever now the renewed formation of antibody has reached a sufficient concentration, it will always find sufficient allergen at the point of vaccination to produce the toxin.

Bloch states that as far as his experience goes, the cutaneous reactions were never positive in cases which did not have a trichophyton infection. We have four cases (Table II) where the reaction was positive, while there was no history obtainable of any previous skin disease. Two of these cases (Nos. 1 and 2) were 39 and 54 years old, both inmates of the Bay View Hospital, and the correctness of their data may be open to doubt. But the two other cases (Nos. 3 and 4) were students, and one of these took special pains to find out whether in his early life a skin affection had been noticed. As far as could be ascertained, in both these cases there had been no skin affection. At present we will refrain from drawing any conclusions from these observations, being satisfied with the statement of the facts as they presented themselves. Of the four cases giving a history of ringworm with negative reaction (Table III), one was a case of Dhobie itch, and this disease is not necessarily due to a trichophyton infection. One case was a man 72 years of age, giving a history of ringworm when young; the other was a

TABLE II.

No History of Trichophytosis and Positive Reaction.

	Age.	History.	Remarks.
1. W. B.	39 years.	Does not know of any scalp, beard or skin disease.	After 48 hours exudation, hardly any inflammation. 48 hours later, red, raised papule about 8 mm. in diameter gradually disappearing within the next week.
2. S. S.	54 years.	Same history.	After 48 hours red, raised papule about 8 mm. in diameter disappearing within a week.
3. Mr. Tr.	25 years.	No known infection with trichophyton. Parents state that he had never any skin affection.	After 24 hours red, raised papule 5 mm. in diameter. During the next days increased in size and intensity, itching, gradually disappearing with scaling. Second vaccination, 69 days later, positive.

4. M. R.	26 years.	No known trichophytosis.	After 24 hours little red and raised papule of about 6 mm. in diameter. 24 hours later about same size but somewhat more intense.

TABLE III.

History of Trichophytosis and Negative Reaction.

	Age.	History.	Remarks.
1. C.	55 years.	20 years ago skin eruption on leg, of short duration. Doctor called it ringworm.	No reaction within 9 days.
2. B.	64 years.	Occasional attacks of ringworm when quite small. Cured at home with green walnuts.	No reaction within 2 weeks.
3. J.	72 years.	Ringworm when young.	No reaction within 2 weeks.
4. M. P.	32 years.	Dhobie itch in Philippines 4 years ago. Very intense. (Never any other trichophytosis.)	No reaction.

man 64 years of age, giving a rather doubtful history of ringworm when quite small; and finally, there is the case of a man 55 years of age, having had a skin eruption on the leg for twenty years, previously diagnosed as ringworm. In these cases the time elapsed between infection and vaccination may have been sufficient to permit the disappearance of the antibodies, even if there should have been a trichophytia profunda, or supposing the skin disease really due to a trichophyton infection, it may have been a more or less superficial one.

With regard to the cases giving a history of a skin affection possibly due to a trichophyton infection with a positive reaction, a few are well substantiated. A detailed discussion of the others would lead us too far. Suffice it to say that more detailed data are desirable.

Dr. B. (Table I, No. 5), giving a definite history of trichophytosis, showed a positive reaction. He was first vaccinated on December 18, 1909, and for the second time on December 20. Twenty-four hours after the second vaccination a faint, indistinct,

red halo was seen, very irregular in shape, surrounding the sharply defined red and raised papule. This halo had disappeared about thirty-six hours after the vaccination. On March 2 the vaccinations were again commenced with the intention of increasing the reactivity as much as possible by repeated vaccinations. Until March 11 Dr. B. was vaccinated four times, and each time the halo formation was pronounced. The vaccinations were made on the right and left arm, twice separately and twice simultaneously. The first vaccination on the left arm was definitely less intense than that on the right, which had been vaccinated before, while the subsequent vaccinations were alike in intensity. On March 14, at 9.30 A. M., Dr. B. was vaccinated on the right thigh with different dilutions of trichophytin; with each dilution four points were made in exactly the same manner described by v. Pirquet in his experiments with tuberculin. From the knee upwards the first point was a control without any trichophytin, the next row was made with a dilution of 1:256, the next with 1:64, the next with 1:4, the next with 1:2, and the last with undiluted trichophytin. In certain intervals the reactions were measured and their condition noted. From the measurements of the reactions with the different dilutions, an average was calculated and the values thus determined were used for the construction of the curves. The reactions were measured by marking their extent in two directions with pen and ink, and measuring the distance between the points thus marked.

The following protocol illustrates the manner of recording. It was taken twenty and one-half hours after vaccination and represents the highest development of the papules noted in our measurements. The order in which the reactions are given is from the inside of the thigh to the outside.

Control 2:2 millimeters.

1/256	4.5:5	3.5:3.5	5:4.5	4:3.5
	Fading; redness diminished; induration diminished.			
1/64	5:5	4.5:4.5	6.5:5	7.5:5
	Tender, raised, red and indurated.			
1/16	7:7.5	7:7	6:6.5	8:8
	Tender, red, raised and indurated.			
1/4	9.5:8.5	8:8	10.5:9.5	8:8.5
	Very tender, much raised and indurated; intensely red.			
1/2	9:8	11:10.5	10:8.5	10:9

Very tender, much raised and indurated; intensely red.

1 12 : 10.5 9.5 : 11.5 11 : 11 10.5 : 10

Very tender, much raised and indurated; intensely red.

All reactions rather well circumscribed; no halo formation.

The following table represents the average measurements of the reactions at different times.

	March 14.				March 15.			March 16.		Mar. 17.
Vaccination 9.30 a. m.	1.30 p. m.	5.30.	9.30.	12 night.	6 a. m.	12 noon.	7.30 p. m.	12 noon	11 p. m.	12 noon.
Hours after vaccination	4	8	12	14½	20½	26½	34	50½	61½	74½
Dilutions.	Average Measurements in mm.									
1 : 256	2	3	4.2	4.2	4.2	3.5	3.5	2.4	2	2
1 : 64	2	2.5	4.4	4.6	5.6	5.6	5.3	2.6	2.8	2
1 : 16	2.2	4	6	6.2	7.1	7.3	13.2*	6 11.7†	5.3	3.6
1 : 4	2.5	4.1	5.8	7.1	8.8	9.2	14.6*	6.2 16.3	5.6	4.3
1 : 2	2.8	4.7	6.9	7.8	9.5	9.1	19.6*	6.9 20.6	7.6	4.6
1	2.1	5.1	8.3	9.8	10.8	9.4	23.9*	6.4	8.4	5.4

The figures marked * in the table represent the measurements of the halo surrounding the papule. The one figure marked † is the average of two areas, those of the other two points of this series having disappeared. In the series 1 : 2 and 1 it will be seen that the measurements of the papule showed again a slight increase after 61½ hours. At this time the elevation, induration and intensity of the redness of the papule had diminished to such an extent that it was very difficult to distinguish the papule from the ill-defined remainder of the halo. These measurements, therefore, include the rest of the halo and were not taken into consideration in constructing the curves of the papules. Between the last measurements of March 15 and the first of March 16, the rather long interval of 16½ hours had elapsed. This omission was due to the fact that with the development of the halo Dr. B. was taken sick.

On March 15, about twenty-four hours after the vaccination, the first signs of a halo around the papule appeared in the series 1 : 2 and 1. During the afternoon this halo became more and more pronounced until in the evening, thirty-four hours after vaccination, the halo from 1 : 16 to 1 had assumed a bright red color, not appreciably differing from the papule, so that the papules could not be measured separately. In 1 : 2 and 1 the halos of the neighboring points were running together at different points, so that the meas-

urements of the halo in one direction are not quite exact. At the same time Dr. B. complained of severe headache, fever, general muscular pains, and particularly pain in the vaccinated leg, com-

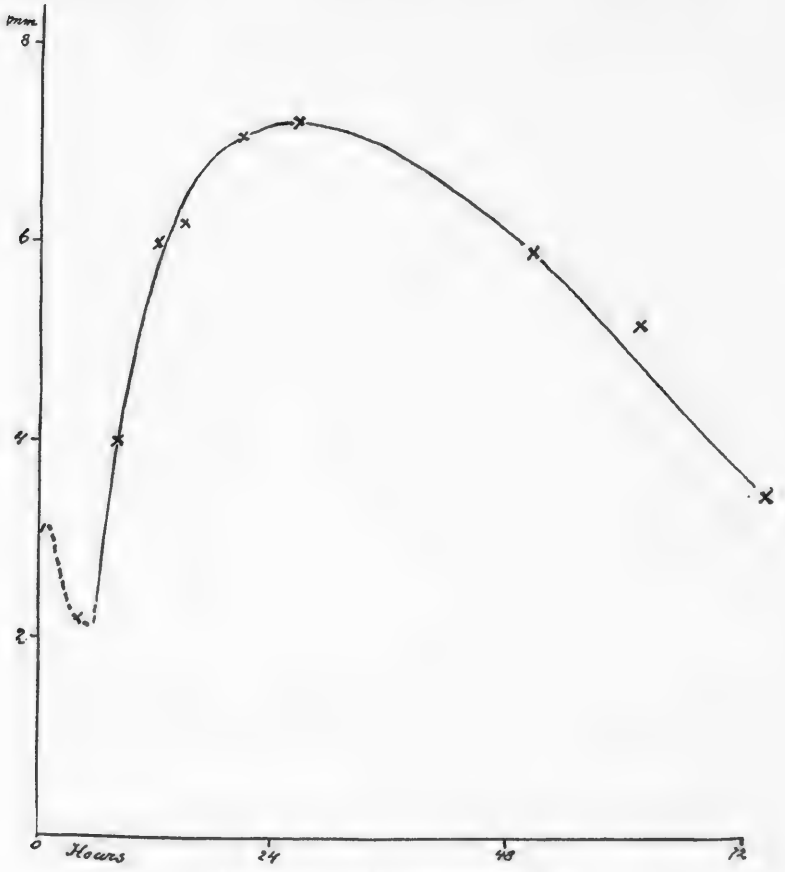

FIG. 1.—Dilution of trichophytin, 1 : 16.

plete loss of appetite, violent throbbing in the head, slow heart action and painful swelling of the right inguinal glands. During the night itching on the field of vaccination added itself to the symptoms. Dr. B. felt too poorly to make any observation during

the night. The next morning he had improved somewhat and noted that there was apparently no change in the size of the reactions, but that the intensity had diminished very considerably. At noon on March 16 the general pains had disappeared almost entirely, as well as the headache, the reactions were less painful and the inguinal glands had diminished but were still tender on pressure. The halos of 1:2 and 1 were rather well defined, though much less red than the papules. In the series 1:4 only two halos were still to be made out and one other was very indefinite. In the series 1:16 only one of the reactions presented a very indefinite halo. In the course of the day Dr. B. recovered completely and the remaining lassitude had left the next day.

The course of the reaction may be best illustrated by the curves, which were constructed according to the method of v. Pirquet. A system of base line and ordinates was marked on quadrille paper, where the base line represents the hours and the ordinates the size of the reactions, and since the size of the reactions corresponds directly with their intensity, the ordinates represent the intensity of the reactions. This system served for the preparation of all the curves, the paper on which the curves were drawn was laid on top of it, and the figures were marked in their proper places. Then the curves were drawn in such a manner that the line passed through as many points as possible. Fig. 1 illustrates the manner in which the curves were drawn. The curve represents the development of the papule with a dilution of 1:16. The control was not measured until twelve hours after the vaccination, when it had reached the level on which it remained; therefore, it was not possible to mark the traumatic reaction which always follows any vaccinations. The beginning of the reaction proper, therefore, cannot be marked with accuracy in the curves. From the close analogy of our curves with those obtained by v. Pirquet in his experiments with tuberculin, it was thought permissible to indicate the traumatic reaction in our curves as though it were analogous to his findings. To indicate that this part of the curves is not based on our figures, it is drawn in broken lines. Comparing our results with those obtained by v. Pirquet in his experiments with tuberculin, we see by a glance at Fig. 2, representing all the curves, that as in the case of

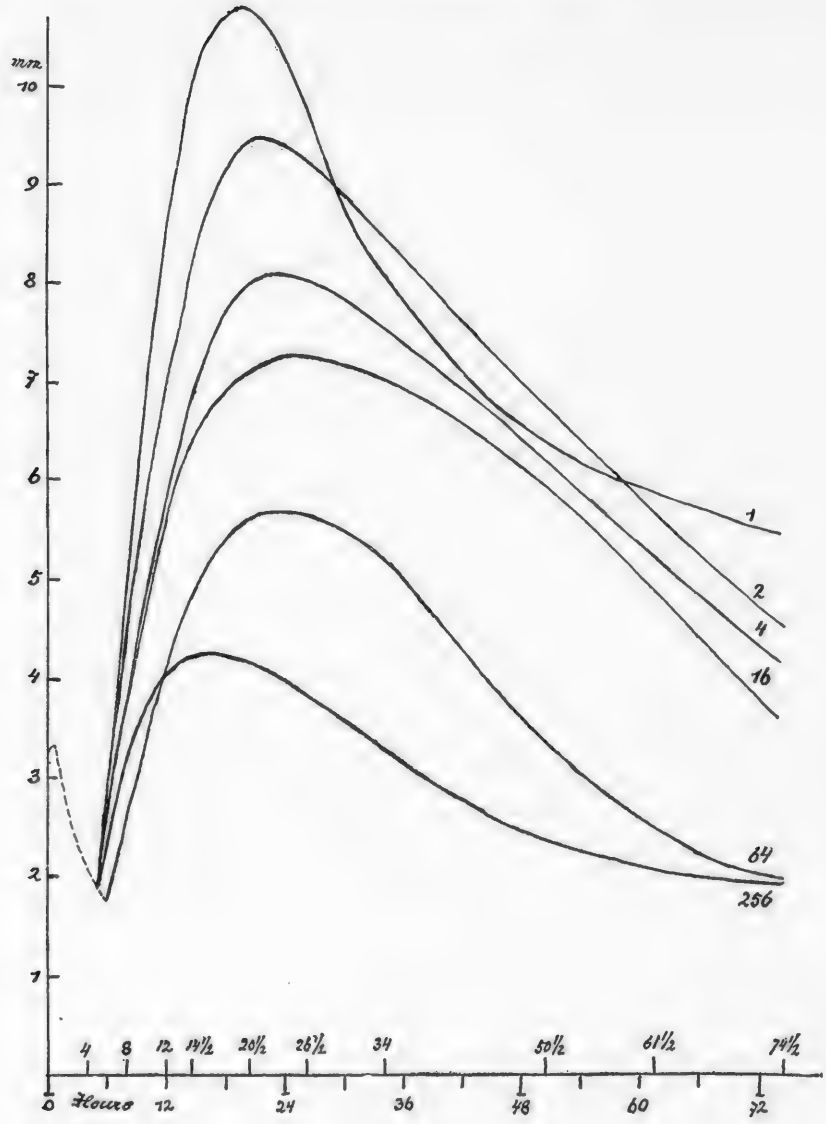
m/m
10
9
8
7
6
5
4
3
2
1
1
2
4
16
64
256
4
8
12
14½
20½
26½
34
50½
61½
74½
0
Hours
12
24
36
48
60
72

FIG. 2.

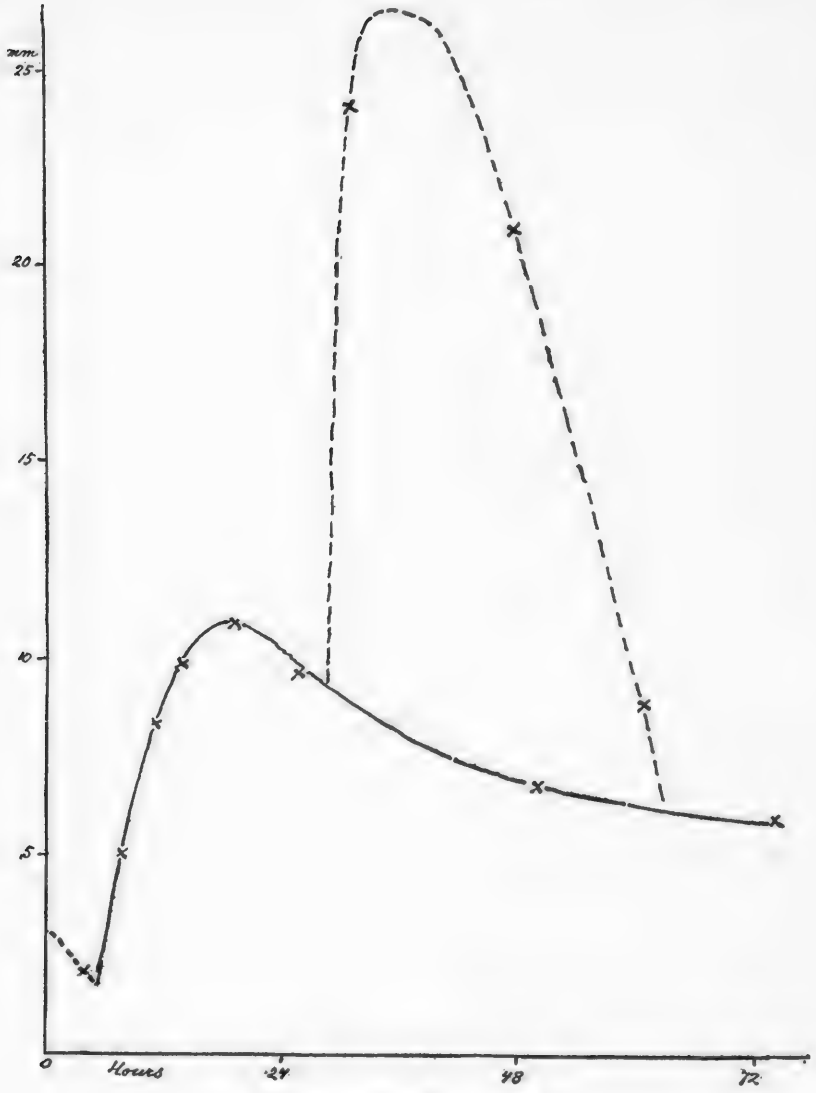

FIG. 3.—Dilution of trichophytin, 1 : 1.

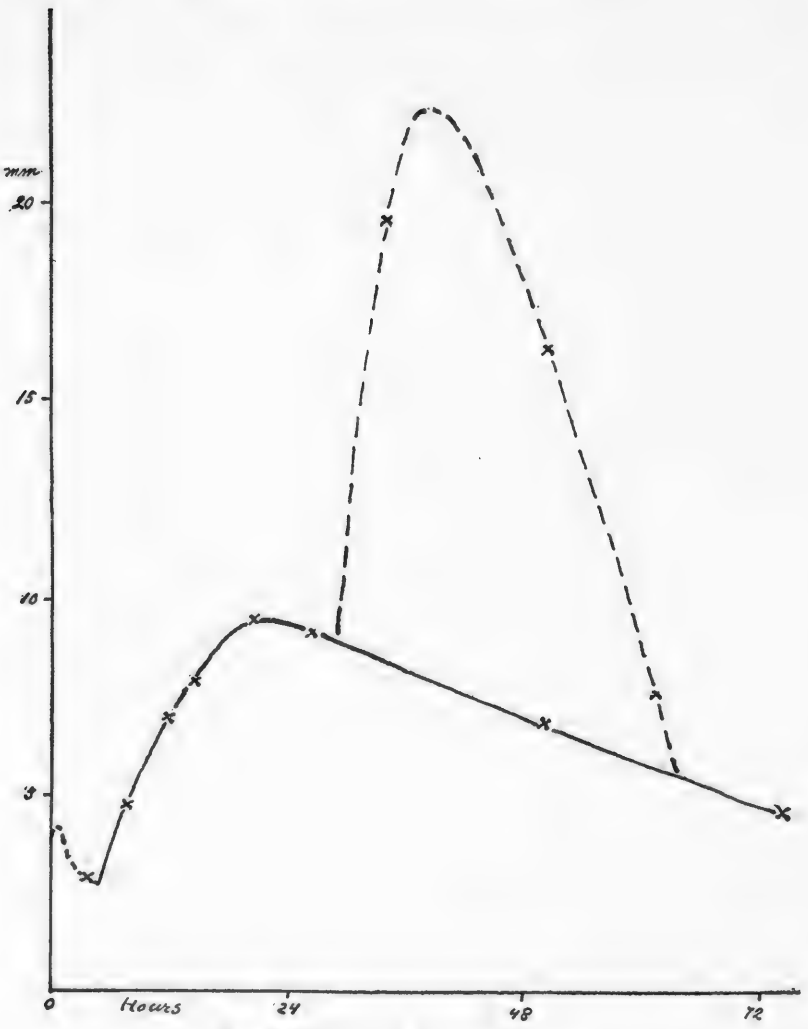

FIG. 4.—Dilution of trichophytin, 1 : 2.

tuberculin, the intensity of the reaction is directly proportional to the concentration of the trichophytin.

The curves in Figs. 3, 4 and 5 are of special interest. The lower

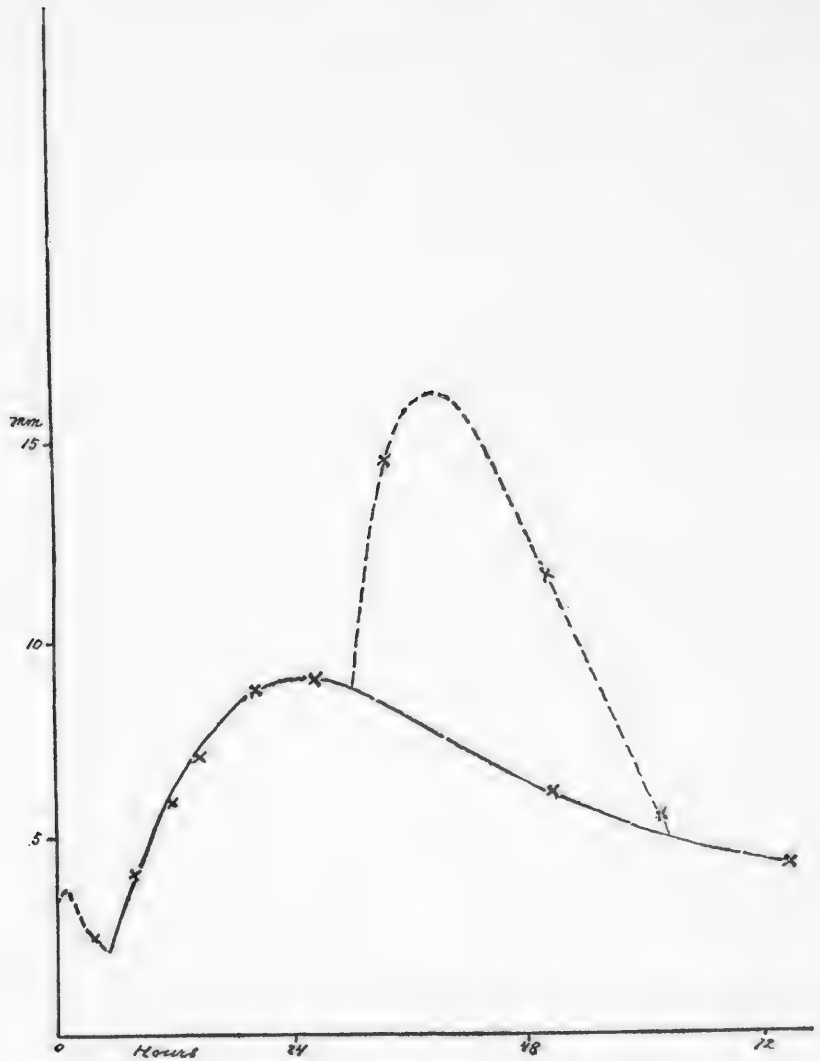

FIG. 5.—Dilution of trichophytin, 1:4.

curve drawn out represents the size of the papule, while the superimposed curve drawn in broken line represents the size of the halo.

The first signs of a halo were seen in some reactions about twenty-four hours after vaccination. Twenty-six hours after vaccination one point in the series 1 : 2 and all of the series 1 showed slight and very ill-defined indications of a halo; after thirty-four hours all the points of the series 1 : 16 were surrounded by an intense halo; after fifty and one-half hours the halos of the series 1 : 16 had disappeared entirely; in the series 1 : 4 only two were still visible and all had practically disappeared after about sixty-one and one-half hours. The starting point of the curve representing the halos, particularly in the series 1 and 1 : 2, should have been a little more advanced. Since the interval between the measurements was rather long, the acme of the curve was not fixed at the time of the measurement, thirty-four hours after vaccination. The time of the onset of the halo again shows a striking analogy with the results obtained by v. Pirquet in the tuberculin vaccination.

Thirty hours and thirty minutes after vaccination he notes: "Now it (the papule) is surrounded by a slightly red halo. The papule itself is smaller than in the previous measurements." This result coincides with those we have obtained as a glance at the curves will show. Here too the beginning of the halo is contemporaneous with the beginning involvement of the papule. Thirty-two hours and twenty-five minutes after vaccination the halo formation in v. Pirquet's experiment was very distinct. After 38 hours the halos were still larger. After 45 hours and 35 minutes the halo could only be seen indistinctly in a few places, but after 53 hours some places still showed a halo presenting in great part a very large (up to 35 millimeters) indefinite reddening. Three days after the vaccination the halos had disappeared entirely. The agreement of v. Pirquet's results with our results is certainly most striking.

Plate XXXIX, Fig. 1, illustrates the appearance of the reactions in our case. It was taken about fifty hours after the vaccination, when the papules were already in the process of involution, rather easily separable from the halos, and the halos were relatively sharply defined, although less sharply than represented in the drawing.

In v. Pirquet's experiment the development of the halos was not associated with a general reaction, at least none was noted. In our case the general reaction was so intimately associated with the development of the halos that we are justified in assuming a connection between these two reactions. A general reaction after tuberculin vaccination has been observed very rarely and no particular attention has been paid in these cases to the halo formation.

The analogy of the halo formation in our trichophytin vaccination with that in tuberculin vaccination consists in the time relation.

For this phenomenon the following explanation may be offered. The introduced trichophytin meets with a certain amount of antibody and the result is the immediate reaction with the formation of the papule. The intensity of the reaction depends, as we have seen, on the concentration of the trichophytin. The intensity of the halo formation also depends on the concentration of the trichophytin, for instance 1:256 and 1:64 do not form any halo at all, and with increasing concentration of trichophytin, the intensity of the halo increases, as may be seen from the table registering the average measurements of the reactions and Figs. 3, 4 and 5. It is conceivable that the concentration of the antibodies is sufficient to combine with all the trichophytin absorbed from the higher dilutions, while it is not sufficient to combine with all the trichophytin of the more concentrated solutions. The trichophytin—the allergen—left over spreads itself in the lymph spaces, a renewed production of antibodies takes place, and with the advent of these, the reaction is again renewed with the formation of the halo. The objection may be raised that under such conditions the first reaction should reach the same degree of intensity from a given dilution on. But the possibility exists that according to the law of mass action with increasing concentration of the trichophytin and uniform concentration of the antibody, a larger amount of the toxic product is formed. If the reaction of antibody and trichophytin follows the law of mass action, and we suppose a relatively large excess of trichophytin, this increased formation of toxic substance would not exclude the possibility that in spite of it with increasing concentration of the solutions a greater excess of trichophytin is left over. This explanation would cover the halo formation in both the tuberculin and the trichophytin reaction.

The intense general reaction of Dr. B. contemporaneous with the halo formation has still to be explained. The rapid development of the halo and its intensity indicate a rapid production of toxin. The larger area over which this reaction was spread may offer a better chance for a quick resorption of the toxic substance, which would then enter the general circulation in greater concen-

tration. The fact that in the tuberculin experiment no general reaction was noted may be due to a lesser toxicity of the toxic substance formed or to a greater individual sensitiveness of Dr. B.

In cowpox vaccination the general symptoms coincide with the formation of the area, while at the same time the involution of the papilla begins. v. Pirquet (12) brought forward strong evidence that the formation of the area and the general symptoms are due to the interaction of the produced antibodies and vaccine lymph with resulting toxic substances. There exists a seeming resemblance between a first cowpox vaccination and the reaction observed in our case. The area formation closely associated with the onset of general symptoms would be analogous to the halo formation, while the papilla beginning its involution at this period would seemingly correspond to the papule. But the phenomena observed at revaccination make it self-evident that this analogy is not a real one. Our type of reaction belongs to the "early reaction." The early reaction in cowpox vaccination leads to the formation of a papule, while the area formation does not take place any more. This papule is analogous to the papule of the tuberculin and trichophytin reaction and it really corresponds to the area of the first vaccinated.

It may be mentioned that the early reaction of cowpox vaccination shows another analogy to the tuberculin and trichophytin reaction, for v. Pirquet (13) showed that its intensity depends on the amount of introduced virus, like that of the tuberculin and trichophytin which is dependent on the concentration of the solutions. In the case of a first vaccination the intensity of the reaction is not dependent on the amount of virus originally introduced.

The formation of the area, as well as that of the papules, depends on the presence of antibodies, but in the first case the production of the antibodies requires some time during which the microorganisms of the lymph multiply and spread themselves to some extent in the surrounding tissue. Before the advent of the antibodies, the allergen increases constantly, and when the antibodies arrive, the concentration of the allergen is sufficient to elicit a violent reaction producing an area with general symptoms. In the early reaction, the organism, due to a previous infection, is already

in possession of a sufficient amount of antibody to react immediately with the introduced allergen, just as in the tuberculin and trichophytin reaction, only perhaps with the difference that at first substances analogous to the tuberculin or trichophytin must be liberated. There is no time left for the multiplication of the microorganisms, that is, for an increase of the allergen. The result is a relatively insignificant reaction with only a papule formation. The formation of the papilla is dependent and proportional to the multiplication of the microörganisms of the lymph. Therefore, it is obvious that there is an essential difference between the papilla and the papule of the early reaction. That nothing corresponding to our halo formation is seen in the early reaction of cowpox vaccination can be easily explained. An early reaction in this case means a sufficient concentration of antibodies to inhibit the growth of the specific microörganisms immediately. The concentration of the antibody is relatively great when compared to that of the allergen, which did not have time to increase, while for the formation of the halo it seems necessary that the active mass of the allergen exceeds that of the antibody.

From our experiment the conditions for the formation of a halo are the following: Sufficient concentration of the antibody to produce an early reaction and excess of introduced allergen. The allergen left over spreads in the lymph spaces, stimulates a renewed production of antibodies, and this renewed production of antibodies proceeds rapidly, resulting in a rapid rise of the reaction. With a change of concentration of the antibodies on one side and that of the allergen on the other, we may naturally expect different degrees of halo formation and changes in its time relation. That some of the trichophytin may remain at the place of vaccination for a long time is seen in the case described, where the reaction did not show itself before the sixth day. According to the conceptions of v. Pirquet, it is very probable that the whole organism participates in the cutaneous tuberculin reaction, although in the great majority of cases the manifestations remain below the threshold of clinical observation. The renewed production of toxic substance in our case may have sufficed to bring these manifestations above the level of clinical observation in a specially sensitive individual.

Our explanation of the halo formation does not seem to agree well with the observation of v. Pirquet in his experiment with tuberculin vaccination. Here all the vaccinations varying from a dilution of 1:512 to undiluted tuberculin showed a halo formation, and even the control participated in the hyperemia. No data are given about the intensity of the halo formation with the different dilutions. The participation of the control itself cannot be explained at present. That the higher dilutions participated in the halo formation may be due to the fact that the tuberculin contains the allergen in greater concentration than the trichophytin.

Unfortunately we did not have the opportunity of testing acute cases of trichophytosis, but from the description of the cutaneous reaction given by Bloch and Massini it seems that the reaction was never as intense as is frequently seen in the tuberculin reaction, as we had the opportunity to see in the inmates of the tuberculosis ward at Bay View. We, therefore, may assume that the concentration of the allergen in the tuberculin is really greater than that of the allergen in the trichophytin. The method of preparation of tuberculin and Bloch's trichophytin would speak also in favor of such a view.

Our explanation of the halo formation is tentative, and we are well aware that it needs further investigations to establish it on a firmer basis.

CONCLUSIONS.

There exists a far-reaching analogy between the cutaneous trichophytin reaction and the cutaneous tuberculin reaction. Both indicate that the organism is the seat of a definite infection or that it has passed through such an infection.

Both may persist for a long time after the active disease has come to rest, indicating that the infection has left the organism in a state of altered reactivity—allergy.

Under certain conditions both may be of diagnostic value, but since the reaction persists for a long time after the infection has passed, the negative reaction may be of greater value, excluding the existence of a specific infection.

The analogy of the trichophytin reaction with the tuberculin reaction is not only limited to the obvious clinical manifestations, but, as in the tuberculin reaction, it can be shown that with uniform concentration of antibody, the intensity of the reaction is dependent on the concentration of the trichophytin.

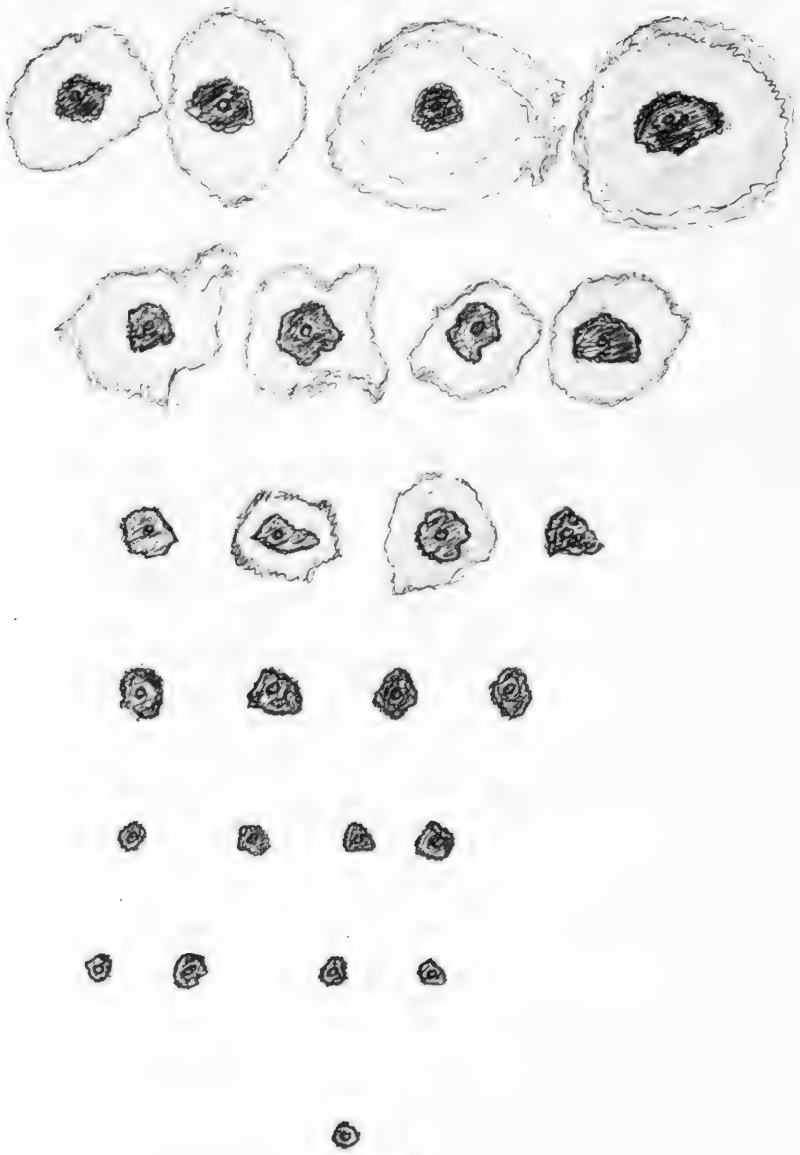

FIG. 1.

A tentative explanation of the halo formation is offered, based on a rapidly renewed formation of antibody stimulated probably by the entrance of a small amount of allergen into the general circulation.

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LATENT LIFE OF ARTERIES.[1]

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Plates XL–XLIII.

I. INTRODUCTION.

A tissue is in latent life when its metabolism becomes so slight that it cannot be detected, and also when its metabolism is completely suspended. Latent life means, therefore, two different conditions, unmanifested actual life and potential life. Unmanifested actual life is a normal stage of the evolution of all organisms, when they progress from general death toward elemental death. As the metabolism is still going on, although very slowly, it is a temporary condition. Sooner or later cadaveric lesions develop which bring about the complete disintegration of the protoplasm. Potential life consists of a suspension of all actual vital processes. The metabolism being suppressed, no cadaveric changes would take place in the protoplasma. Tissues in a condition of potential life could be preserved outside of the body for an indefinite period of time.

Since the discovery by Loewenhoek, many observers have studied the conditions and the nature of latent life. Their work was of great biological and metaphysical importance, but it did not seem that their observations would ever be of practical interest. Nevertheless, it happens to-day that the evolution of surgery leads to the use of latent life in human therapeutics. In several kinds of operations on blood-vessels, nerves and even the ureter, the graft of vascular segments can be employed. Human vessels must be used, because autoplastic and homoplastic transplantations are more successful than heteroplastic transplantations. Autoplastic grafting of arteries is not possible, since every large artery is necessary to the organism. Homoplastic grafting only can be used. The graft must be taken from an amputated limb, or from

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the fresh cadaver of an executed criminal or of a man killed by accident. But such an opportunity will certainly not occur precisely when a graft is needed for an operation. The grafts, therefore, must be kept in storage and be ready for use when necessary. Such a preservation of vessels outside of the body can be obtained by placing them in a condition of latent life.

What form of latent life can be used for preserving tissues of mammals outside of the organism? Although unmanifested actual life is only a slow progress towards elemental disintegration, it would be of practical value. It would permit us to preserve the tissues for some time in such a condition that they could be revived if given back their normal physico-chemical conditions. A tissue, immediately after its extirpation from the body or after death, is still living. Its metabolism is going on slowly, while the microbian and autolytic fermentations begin which sooner or later bring about elemental death. There is a period during which the cadaveric lesions are still so slight that they can be interrupted, if the tissues are placed again in their normal condition. The length of the period intermediate between general death and protoplasmic destruction varies according to the nature of the tissues, the temperature and a great many other factors. It is well known that epidermis or periosteum can be kept for a long time outside of the body and be grafted successfully. Sixty years ago, Paul Bert (1) showed that the transplantation of rats' tails, preserved for several days in a small quantity of confined air, at a temperature not higher than + 12° C., gave positive results. Lately, Jolly (2), examining a few drops of triton's blood, which had been preserved for fifteen days in cold storage and then put back at normal temperature, saw the red blood corpuscles undergoing indirect division. Ehrlich (3) has demonstrated that pieces of tumor which have been frozen for a long time grow again when transplanted. Preservation of arteries in unmanifested actual life would not be perfect, because it retards only and does not suppress the occurrence of cadaveric lesions. It may be, nevertheless, of practical value in surgery.

The ideal method would be to place the vessels in a condition of potential life. The metabolism being completely stopped, the duration of the period of preservation could be indefinite. Many

physiologists do not admit the possibility of the complete suspension of metabolism. They believe that when Doyère (4) dried a rotifer, heated it at 100° C., and revived it afterward, or when Paul Bert grafted successfully upon a rat a rat's tail which had been dried in vacuum and heated at 100° C., the metabolism of these tissues had not been interrupted. It seems, nevertheless, that under certain conditions metabolism can be suspended. Becquerel (5) has preserved seeds for months in a vacuum, at temperatures as low as — 190° C. and — 253° C., and germination occurred afterwards. It seems reasonable to believe that protoplasm can be placed in such a condition that life does not exist actually, but merely potentially. Gautier thought that protoplasm can remain lifeless until it is given its normal physico-chemical condition, like a clock waiting in absolute immobility for the motion which starts its mechanism. Potential life can probably exist in seeds. But it is improbable, although not impossible, that tissues of mammals can be placed in a condition of potential life without undergoing a fatal disintegration.

I began in 1906 some experiments on the preservation of arteries in cold storage (6). It was found immediately that a dog's carotid artery could be preserved outside of the body at a low temperature for several days and transplanted without suffering degeneration of its muscle fibers. Afterwards, I studied more extensively the different methods which can be used in the preservation of vessels in latent life and their biological and surgical results.

II. TECHNIQUE.

The experiments were performed on dogs. The animals were etherized not only during the operation but also during the shaving and the preparation of the skin and the post-operative examination of the vessels. They were operated on by the methods used on human patients.

Each experiment was composed of four stages: extirpation of arteries, preservation, transplantation and examination of the results.

1. *Extirpation of Arteries.*—Segments of common carotid artery were extirpated from medium-sized dogs during life or a short

time after death by ether or chloroform. The arteries were rapidly exposed, dissected, resected and cut in several pieces about three or four centimeters long. They were then washed in Locke's solution and placed in sterilized glass tubes. The extirpation and the handling of the vessels were made under rigid asepsis.

2. *Preservation of Arteries.*—In a first set of experiments, the arteries were killed in order that the evolution of dead arteries and of arteries in latent life could be compared. In a second set, it was attempted to preserve the arteries by freezing and drying. In a third set the arteries were merely preserved in cold storage.

(*a*) *Preservation of Dead Arteries.*—The arteries were killed by being placed into a sealed tube with a few drops of Locke's solution and heated at 80° C. for ten minutes, or by being immersed in glycerin, or in a 2/100 solution of formalin. Afterwards, the formalined vessel was washed in a diluted solution of ammonia and in Locke's solution.

(*b*) *Preservation of Arteries by Freezing or by Drying.*—The tubes containing the arteries were placed in a refrigerator, the temperature of which was oscillating a little above and below — 3° C. The vessels were kept at this temperature for several days. A few hours before the transplantation, the tubes were removed from the first refrigerator and put into another one about at the freezing point. Two hours after, they were taken to the laboratory at a temperature of about + 29° C.

In the preservation by drying, the arterial segments were sealed in a test tube, the bottom of which was filled with calcium chloride. After a few hours, the arteries shrank and became yellowish and, finally, hard and dried like a strand of catgut. They could be preserved in that condition for an indefinite period of time, at the temperature of the laboratory or in cold storage. In one experiment, the tube was placed for several minutes in boiling water. Two hours before the transplantation, the arteries were removed from their tubes and placed in Locke's solution. Progressively, they were imbibed by the fluid, the lumen became visible, the yellowish color disappeared, and after an hour or an hour and a half, they had regained their normal appearance. From a gross anatomical standpoint, they looked exactly like vessels freshly extirpated from an animal.

(*c*) *Preservation of Arteries in Cold Storage.*—The arteries were placed in glass tubes filled with salt solution, Locke's solution, humid air, vaselin, serum or defibrinated blood. The tubes were sealed and deposited in cold storage. Until March, 1908, the temperature of the refrigerator was often a little above the freezing point, but was also oscillating from — 2° or — 3° to + 12° or + 15°. The apparatus was unreliable. Since March, 1908, I have used an ice box, the temperature of which is constantly between 0° and + 1° C.

3. *Transplantation of the Arteries.*—The arterial segments were removed from the tubes, washed in Locke's solution, and soaked in vaselin. One of the carotid arteries of a dog was dissected and cut, and the preserved segment interposed between and sutured to its ends by the ordinary method. Rigid asepsis was employed in these operations. The degree of asepsis varied according to different changes in the organization of the laboratory. From October, 1906, to October, 1908, the degree of asepsis was generally high. From October, 1908, to January, 1909, it was lower and some non-suppurative infections were observed. After January, 1909, asepsis became excellent.

4. *Examination of the Results.*—The results were examined clinically and anatomically some time after the operation. After a few weeks or a few months the neck of the dog was reopened, the vessel was dissected and the conditions of the circulation were examined. It is important to observe the transplanted segment while the blood is circulating. Its morphology is often different, whether it is functionating or not. Some of the animals were examined in that manner several times. When the time which had elapsed since the operation was considered sufficient, the specimen was removed and examined from a gross anatomical standpoint. Sections of the transplanted arteries were fixed in Zenker's fluid, embedded in paraffin, sectioned and stained with hematoxylin-eosin, Weigert's elastic tissue stain, and with von Gieson's method, when necessary.

III. EXPERIMENTS.

The experiments are divided in three groups: (*a*) transplantation of dead vessels, (*b*) transplantation of frozen or dried vessels, (*c*) transplantation of vessels preserved in cold storage.

(*a*) *Transplantation of Dead Vessels.*—Three experiments were performed. The arterial segments were killed by formalin, glycerin and heat.

Experiment 1. Artery Killed by Formalin.—Small brown dog, No. 313. November 4, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on November 2, 1908, put in a 2/100 solution of formalin for two days; washed in a weak solution of ammonia, in Locke's solution, and placed afterwards in vaselin. *Microscopical examination.*—Section 141. Artery normal. November 12. No pulsations. Opening of the neck. Obliteration. Marked reaction of the connective tissue. *Microscopical examination.*—Section 166 (Plate XL, Fig. 1). No intima. Media: elastic fibers stretched, no muscular nuclei, a few debris of nuclei in outer layers of the media. Adventitia very much thickened.

Experiment 2. Artery Killed by Glycerin.—Brindle female dog, No. 307. October 27, 1908. Transplantation on the right carotid artery of a segment of carotid preserved in glycerin, and washed in Locke's solution. *Microscopical examination.*—Section 131. Artery almost normal. Nuclei of the muscle fibers retracted and many of them uniformly stained. November 4, 1908. No pulsations. Segment obliterated. Very marked reaction of the surrounding connective tissue. Segment extirpated. *Microscopical examination.*—Section 143. No intima. Media: elastic fibers almost normal. All the muscular fibers have completely disappeared and are replaced by amorphous substance. Very marked thickening of the adventitia.

Experiment 3. Artery Killed by Heat.—Very young white and black male dog, No. 309. October 28, 1908. Transplantation on the right carotid artery of a segment of carotid, extirpated from a dog on October 26. The segment was placed in a sealed tube, the atmosphere of which was humidified by a few drops of Locke's solution, and then placed in cold storage. On October 27, the tube was placed in hot water for 8 minutes, during which time the temperature went from 85° C. to 70° C. Afterwards it was deposited again in cold storage. The examination made on October 28 showed the artery small and retracted, and the muscle nuclei darkly stained and deformed. November 12, 1908. Dissection of the artery. No pulsations in the transplanted segment. Obliteration. Adhesion to the vagus. Marked reaction of the connective tissue. Resection of the artery. *Microscopical examination.*—Section 161. Artery dilated. No intima; lumen filled with thrombus. Media: elastic fibers stretched, broken and partly destroyed. Muscle fibers have disappeared. However, a few debris of nuclei are seen in the outer layers of the media. Adventitia very much thickened.

(*b*) *Transplantation of Dried and Frozen Arteries.*—Four transplantations of dried arteries and two transplantations of frozen arteries were performed.

Experiment 4. Dried Artery.—Dog No. 315. November 5, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on October 26. Immediately after extirpation, the artery was put in a

sealed tube half filled with calcium chloride. After a few hours, the artery became dry and assumed the appearance of a piece of catgut. It was then preserved at the temperature of the laboratory. On November 4, the tube was broken and the artery placed in a tube filled with slightly alkaline Locke's solution. On November 5, the vessel was found with its normal appearance, color and consistency. It was put in normal Locke's solution, in vaselin and grafted into the dog. November 12. Dissection of the carotid. Reaction of the connective tissue around the vessel. Obliteration. *Microscopical examination.*—Section 163. No intima; lumen filled with organized thrombus. Media: elastic frame-work normal. No muscular fibers. Adventitia thickened and infiltrated by leucocytes.

Experiment 5. Dried Artery.—Yellow, male, middle-aged bull, No. 320. November 11, 1908. Transplantation on the left carotid artery of a segment of carotid extirpated from a dog on November 5. The segment has been desiccated in a tube of calcium chloride and placed for one hour in Locke's solution just before the transplantation. It resumed its normal appearance. After transplantation and reëstablishment of circulation the vasa vasorum are immediately injected with blood. Normal color, caliber and consistency. *Histological examination.*—November 25. No pulsation on the left side. November 30. Opening of the neck. Obliteration of the transplanted segment. *Microscopical examination.*—Section 184. Lumen filled with thrombus. Intima present in some places and very much thickened. Media dissected in several places by infiltration of red blood corpuscles. Elastic frame-work normal. In many places, the muscular fibers have completely disappeared. In one point of the media, close to the adventitia, a number of muscular fibers are still normal. Adventitia a little thickened.

Experiment 6. Dried and Heated Artery.—Yellow, male, middle-aged bull, No. 320. November 11, 1908. Transplantation on the right carotid of a segment of carotid artery extirpated on November 5, 1908, desiccated in a tube of calcium chloride, heated for 12 minutes at 100° C., and placed for one hour in Locke's solution, just prior to the transplantation. *Microscopical examination.*—Section 154. Artery apparently normal. But under high power, muscle fiber nuclei appear to be deformed. November 25. Normal pulsations. November 30. Opening of the neck. Normal pulsations. Normal appearance of the vessel. No modification of caliber of the transplanted segment. Extirpation of the upper part of the segment and reëstablishment of the circulation by a segment of jugular vein. *Microscopical examination.*—Section 185. No intima. Media: thinner than normal, elastic fibers normal, no muscular fibers. However, a very few elongated nuclei are seen in the more external layers, close to the adventitia. Adventitia exceedingly thickened, about twice and three times thicker than the media, according to the location.

Experiment 7. Dried Artery.—White, black and yellow bitch, No. 324. November 20, 1908. Transplantation on the right carotid artery of a segment of carotid artery extirpated on November 16, 1908, and dried on calcium chloride. *Microscopical examination.*—Section 177. No intima. Adventitia normal. Media: elastic fibers normal, nuclei of muscular fibers more irregular and uniformly stained. November 23. Normal pulsations. June 8, 1909. Dog died at the farm. *Macroscopical examination.*—Dissection of the carotid. No ad-

hesions to the vagus. The anastomoses are easily seen as very narrow brownish lines. Transplanted segment has same color, caliber and consistency as normal carotid. *Microscopical examination.*—Section 236. Intima as thick as the media in some points, composed of layers of connective tissue cells with elongated nuclei and infiltrated by a great many exceedingly thin elastic fibers, especially in its inner parts. Media: interna elastica normal (Plate XL, Fig. 2), no muscular fibers. However, a few elongated muscles are seen between the outer elastic layers. Adventitia a little thickened.

Experiment 8. Frozen Artery.—White and black male dog, No. 149. July 11, 1907. Transplantation on the carotid artery of a segment of carotid extirpated from a dog on July 8. The segment was put in a refrigerator at the temperature of —3° C. immediately after the extirpation. It was removed from the refrigerator on July 11 at 6 A.M., and put into a refrigerator at a temperature of + 1° C. At 9:10 A.M. it was brought to the operating room at a temperature of 29° C. July 16. No pulsations. Neck opened and artery dissected. Obliteration of the lower anastomosis. Upper anastomosis normal. Wall of the transplanted segment apparently normal. *Microscopical examination.*—Section 51. Transverse section at the level of the middle part of the transplanted segment. No intima. Adventitia very much thickened. Around small pieces of silk, very large foci of leucocyte infiltration. Media deeply modified. In the internal portion all the muscular fibers have disappeared. Between the laminæ of the elastica, a few leucocytes are seen. In the outer layers of the media, a few deformed or uniformly stained muscle cell nuclei still persist. Longitudinal section at the level of the upper anastomosis. The carotid of the host is almost normal, although its intima is infiltrated by leucocytes. Between the ends of the normal and transplanted carotids, there is a large scar. All the muscle fibers of the transplanted carotid have disappeared. A few leucocytes and debris of muscle fibers are seen between the elastic tissue fibers. Typical non-suppurative infection.

Experiment 9. Frozen Artery.—No. 152. July 16, 1907. Transplantation on the left carotid artery of a segment of carotid preserved since July 11, by the method used in Experiment 8. July 25. Normal pulsations. October 1. No pulsation. October 9. No pulsations. Extirpation of the carotid artery. Complete occlusion of the transplanted segment.

(*c*) *Transplantation of Arteries Preserved in Cold Storage.*—The arteries kept in cold storage were preserved in different media. The experiments are divided in several classes, according to the nature of the fluid.

In three experiments the vessels were kept in normal saline solution or in Locke's solution.

Experiment 10. Salt Solution.—White bitch, No. 5. October 31, 1906. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on October 30. The segment was preserved into a tube of 10/1000 sodium chloride solution, and placed in cold storage. The temperature was irregular, a little above the freezing point. December 4. Dissection of the

artery. Normal pulsations on and above the transplanted segment. Adhesions to the vagus. Resection of the segment. The lumen is slightly enlarged, the wall thickened and the anastomoses normal. *Microscopical examination.*—Section 5. Adventitia thickened. Media: muscular fibers of the inner layers of the media have completely disappeared. Between the elastic laminæ, which are normal, there are a number of mononuclear leucocytes. The middle and outer layers of the media are composed of elastic laminæ and of muscular fibers. In the middle part of the media, the nuclei of the muscular fibers are deformed and surrounded by a few leucocytes. No intima. A thin coat of fibrin is adherent to the interna elastica (Plate XLI, Fig. 3).

Experiment 11. Salt Solution.—Small, male fox terrier, No. 8. November 5, 1906. Transplantation between the central end of the right carotid and the peripheral end of the right external jugular vein of a segment of carotid extirpated from a dog on October 30 and preserved in sodium chloride solution and in cold storage. December 5, 1906. Pulsations and thrill of the external jugular. December 19, 1906. No pulsations, no thrill. Dissection of the vessels. Transplanted segment surrounded by thick connective tissue. Very strong adhesions to the anterior edge of the sterno-mastoideus muscle. Segment occluded by a clot adherent to the upper anastomosis. Lower anastomosis normal. *Microscopical examination.*—Section 168. Adventitia very much thickened. Intima thickened. Media: elastic fibers normal, muscle fibers generally normal. Some of the nuclei are irregular and deformed.

Experiment 12. Locke's Solution.—Black bitch, No. 80. March 6, 1907. Resection of a short segment of the left carotid. Transplantation of a segment of carotid extirpated on February 26, and preserved in Locke's solution. May 3. Dissection of the artery. Normal circulation. No adhesions to the vagus nerve. Appearance of the artery entirely normal. Almost no evidence of the previous operation. May 15, 1908. Dissection of the artery. Slight adhesions to the vagus. Normal circulation. The location of the transplanted segment is hardly recognizable. Resection of the carotid. The location of the transplanted segment becomes evident, because the normal artery contracts itself, while the segment does not. Anastomoses normal, almost invisible. Intima smooth and glistening. *Microscopical examination.*—Section 126. Adventitia normal. Media: the normal elastic frame-work has practically disappeared. It is replaced by a great many small elastic fibers, diffused through the media. No muscular fibers. Intima irregularly thickened, composed of dense connective tissue, covered by endothelium.

In five experiments, the arteries were kept in sealed tubes, the atmosphere of which was humidified by a small quantity of Locke's solution.

Experiment 13. Confined Humid Air.—Yellow, male cur, middle-aged, No. 208. January 14, 1908. Transplantation on the right carotid artery of a segment of carotid artery extirpated from a white bull bitch, No. 204, on January 10, 1908, and kept in a sealed glass tube, the atmosphere of which was humidified by a few drops of Locke's solution. The tube was put into a refrigerator, the temperature of which oscillated between 0° and about 10° C.

February 20, 1908. Dissection of the carotid. Normal pulse. Dog sent to the farm. May 25, 1909. Dog normal. July 22, 1909. Killed in a fight with other dogs. *Microscopical examination.*—Section 244. Upper part of the trans-

planted segment. Intima very much thickened. In one place, it is almost as thick as the media. It consists of two coats of connective tissue cells, the internal coat being composed of transverse fibers, the external of longitudinal fibers. In the inner part of the intima, several layers of elastic fibers have developed. Media: elastic fibers normal, but more closely distributed than normally. A few nuclei of muscle fibers in the outer layers of the media. The largest part of the media is completely deprived of muscle fibers. The interelastic spaces are filled with amorphous substance. Adventitia thickened.

Experiment 14. Confined Humid Air.—White and black young male fox terrier, No. 216. January 24, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on January 10, from Dog No. 204. *Microscopical examination.*—Section 75. Many muscular fibers, nuclei deformed and uniformly stained. November 14, 1909. Dog in excellent condition. Dissection of the right carotid. Slight adhesions to the vagus. Normal pulsations. Caliber of the carotid normal. By a close examination, the transplanted segment can be found. The location of the anastomoses can also be detected. Resection of the carotid. The transplanted segment becomes immediately very apparent because it does not contract itself, while the normal part of the carotid does. Internal surface of the transplanted segment and anastomoses are smooth and glistening. *Microscopical examination.*—Section 257. Intima is composed of dense connective tissue and is very much thickened. In a few places it is thicker than the media. Media is composed of its normal elastic framework and of an amorphous substance. All the muscle fibers have disappeared. Adventitia thickened.

Experiment 15. Confined humid air.—Male bull dog, No. 265. April 1, 1908. Transplantation on the right carotid of a segment of carotid extirpated from a male dog on March 10. Temperature of the refrigerator oscillating between $-2°$ C. and $+10°$ C. May 6, 1908. Dissection of the carotid. Artery is obliterated. Section 102. Lumen of the vessel filled with organized thrombus. Adventitia thick and well vascularized. Media has kept its normal thickness. Elastic fibers and interna elastica normal. Nuclei of muscular fibers normal. Many of them have still their vesicular appearance and their regular shape. Some others are irregular.

Experiment 16. Confined Humid Air.—White, female fox terrier, No. 267. April 2, 1908. Transplantation on the left carotid, of a segment of carotid extirpated from a male dog on March 10. April 9. Dissection of the artery. Normal pulse. Normal size and consistency of the transplanted segment. Its caliber is slightly larger than normal. October 15, 1908. Dog chloroformed. Dissection of the carotid arteries. Arteries are absolutely normal. No evidence of the previous operation. Anastomosis cannot be located. After longitudinal opening of the vessel, it is possible to locate the anastomosis and the transplanted segment, which has become absolutely identical with the other parts of the vessels (Plate XLI, Fig. 4). Section 260. *Microscopical examination.*—Longitudinal section at the level of the upper anastomosis. Intima is very much thickened, almost as thick as the media. It is composed of elongated cells, the nuclei of which assume the appearance of muscle cell nuclei. Media is composed of elastic frame-work, amorphous material and cells with elongated nuclei. Adventitia normal.

Experiment 17. Confined Humid Air.—Male dog, No. 268. April 3, 1908. Transplantation on the left carotid of a segment of carotid extirpated from a young male dog on March 16. May 7, 1908. Segment obliterated. *Microscopical examination.*—Section 101. Lumen filled with an organized thrombus. Adventitia thickened and vascularized. Elastic laminæ destroyed in several places. Muscular fibers normal. Infiltration of leucocytes in the media and the adventitia, which is very much thickened.

Experiment 18. Confined humid air.—Long-haired, black, male dog, No. 286. May 28, 1908. Transplantation on the left carotid of a piece of dog's carotid artery, extirpated on March 16, 1908. For several weeks, the temperature of the cold storage was irregular, going below the freezing point from time to time. October 15, 1908. Animal etherized. Carotid artery exposed. No evidence of anastomoses. By a very close dissection of the artery, two very indistinct lines are seen on the surface of the wall. The diameter and the appearance of the transplanted segment are identical with those of the carotid artery. The adaptation of the transplanted segment to the artery is absolutely perfect. The artery is extirpated. As soon as the circulation stops, the limits of the segment become evident, because the artery contracts its wall while the transplanted segment does not. *Microscopical examination.*—Section 122. Intima very much thickened. At one point, it is thicker than the media. No endothelium, except in a few places. Media thinner than normal. Interna elastica normal. Other laminæ are indistinct. Media is composed chiefly of diffuse elastic fibers. No muscular fibers. Adventitia a little thickened (Plate XLII, Fig. 5).

Experiment 19. Confined Humid Air.—White and yellow, male fox terrier, No. 291, October 2, 1908. Graft on the left carotid artery of a segment of dog's carotid artery, extirpated on March 16, 1908.

The segment is a little modified. Its wall is softer and thinner than usual. It looks like a venous wall. Nevertheless, after reëstablishment of the circulation, the vasa vasorum are immediately injected with blood. They are larger than normal without lateral hemorrhages. October 5. Neck of the dog enlarged. Incision. Hematoma. Ablation of the clots. Large hemorrhage. Slight necrosis of the wall near the upper anastomosis. Ligature of the carotid and extirpation of the transplanted segment.

Experiment 20. Confined Humid Air.—Long-haired yellow dog, No. 293. October 3, 1908. Transplantation on the carotid of a segment of dog's carotid extirpated on March 16. October 15. Segment very adherent to the vagus. Distended and obliterated. The wall is dark, but has kept in some places its normal appearance. The wall is necrosed. The vessel is extirpated and the animal recovers.

Experiment 21. Confined Humid Air.—White and yellow, male fox terrier, No. 292. October 15, 1908. Transplantation on the right carotid of a segment of carotid extirpated from a dog on September 29. *Microscopical examination.*—Section 120. Muscle cells nuclei deformed, retracted and uniformly stained. October 19, 1908. Dissection of the carotid. Normal pulsations. No adhesions of the segment to the vagus. Resection of a small part of the transplanted segment. Suture and reëstablishment of the circulation. *Microscopical examination.*—Section 124. No intima. Media: elastic fibers normal. All muscular fibers, without exception, have disappeared. Adventitia: a few

debris of nuclei. No wandering cells. On the external side of the adventitia, connective tissue of the host forms, in some places, a coat infiltrated with leucocytes.

Experiment 22. Confined Humid Air.—Medium-sized, white fox terrier, No. 310, October 29, 1908. Transplantation on the right carotid artery of a segment of carotid extirpated on October 22. *Microscopical examination.*—Section 134. No intima. Media: many muscle fiber nuclei uniformly stained. Adventitia normal. November 12, 1908. Marked reaction of the connective tissue around the transplanted segment. Segment obliterated. *Microscopical examination.*—Section 165. No intima: lumen filled with thrombus. Media: elastic fibers normal. A great many nuclei of the muscular fibers are normal. Many polymorphonuclear leucocytes in the outer coats. Adventitia very much thickened and infiltrated by leucocytes.

Experiment 23. Confined Humid Air.—Young, white and black, male dog, No. 309. October 28, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated on October 26. *Microscopical examination.*—Section 132. Normal artery. November 12. Non-suppurative inflammation of the wound. Marked reaction of the connective tissue around the artery. Adhesion to the vagus. *Microscopical examination.*—Section 162. Intima and media absolutely normal. Adventitia thickened (Plate XLII, Fig. 6).

Experiment 24. Confined Humid Air.—Small, brown, male dog, No. 313. November 4, 1908. Transplantation on the left carotid artery of a segment of carotid preserved since November 2, 1908. *Microscopical examination.*—Section 142. Nuclei of many muscle fibers deformed. November 12. Opening of the neck. Non-suppurative infection. Marked reaction of the connective tissue around the vessel. Segment obliterated. *Microscopical examination.*—Section 167. Thrombus: media infiltrated by leucocytes. Many muscle fibers have disappeared. Many also are still normal. Elastic frame-work normal. Adventitia thickened and infiltrated by leucocytes.

Experiment 25. Confined Humid Air.—Small-sized, yellow dog, No. 302. October 21, 1908. Transplantation of the left carotid of a segment of dog's carotid extirpated on September 29, 1908. *Microscopical examination.*—Section 125. No intima. Media: elastic fibers normal. Nuclei of the muscular fibers retracted. Many of them are uniformly stained and have lost their vascular appearance. November 25, 1908. Dissection of the artery. No adhesions. No reaction of the connective tissue. Normal circulation. Extirpation of the segment, which is harder and whiter than normal. *Microscopical examination.*—Section 180. Adventitia normal. Intima very much thickened and composed of elongated connective tissue cells. Media partly composed of an amorphous substance, highly refractive, which is broken in several places. This substance is disposed in two distinct bands. Between them, there are some normal muscular fibers.

Experiment 26. Confined Humid Air.—Yellow and white, young, male dog, January 5, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on February 4, 1909. The color of the segment is yellowish. It flattens more easily than normally. After reëstablishment of the circulation, the vasa vasorum are immediately filled with blood. January 6, 1909. Dog is found dead. *Autopsy:* diffuse broncho-pneumonia.

Experiment 27. Confined Humid Air.—Yellow, male dog, No. 326. November 25, 1908. Transplantation on the left carotid artery of a segment of carotid artery extirpated from a living young dog on November 9, 1908. January 21, 1909. Opening of the neck. Transplanted segment has same caliber and consistency as normal vessel. Its color is a little whiter. Normal pulse. Extirpation of a piece of transplanted segment and end to end suture of the artery. *Microscopical examination.*—Section 181. Media thinner. Adventitia and intima very much thicker. Adventitia formed of two parts: internal, composed of irregular connective and elastic fibers; external, composed of regularly disposed large connective tissue fibers. Media composed of elastic fibers of normal appearance but more closely distributed on account of the disappearance of almost all the muscular fibers, which are replaced by an amorphous substance. In the outer and inner layers of the media, a few muscular fibers can be detected. Some nuclei are normal, some others have lost their vesicular appearance, or are broken and very irregular in shape. Intima elastica normal. Intima very much thickened and covered with endothelium. Lumen of the vessel about same size as before transplantation. October, 1909. Opening of the neck. Normal circulation. Dog sent back to farm.

In ten experiments, the arteries were preserved in defibrinated blood or serum.

Experiment 28. Defibrinated Blood.—Dog No. 66. February 18, 1907. Resection of a small segment of aorta. Graft between the ends of a segment extirpated on February 15, and preserved in defibrinated blood and in cold storage. Suture by patching of the inferior mesenteric artery on the wall of the transplanted segment. February 23. Normal circulation. Extirpation of the aorta and transplanted segment. Anastomoses normal. Very thin coat of fibrin on a part of the wall of the transplanted segment, and on the line of suture of the upper anastomosis.

Experiment 29. Serum.—Male fox terrier, No. 310. October 29, 1908. Transplantation on the left carotid artery of a segment of dog's carotid extirpated on October 27, 1908 and preserved in dog's serum. November 12, 1908. Marked reaction of the connective tissue. Adhesions to the vagus. No pulsations. Obliteration. *Microscopical examination.*—Section 164. No intima. Media: elastic framework and muscular fibers entirely normal. Adventitia a little thickened and infiltrated with round cells.

Experiment 30. Defibrinated Blood.—Yellow, black and white dog, No. 378. January 26, 1909. Transplantation on the left carotid of a piece of carotid preserved in defibrinated blood since January 22, 1909. Dog is sent to the farm a few days after the operation. Died of pneumonia on February 15, 1909. *Macroscopical examination.*—No reaction of the connective tissue. No adhesions to the vagus. Caliber, color and consistency of the transplanted segment normal. *Microscopical examination.*—Section 216. No intima. Media: elastic framework normal. The muscle fibers of the inner layers have disappeared. The fibers of the middle and outer layers are normal. Adventitia a little thickened.

Experiment 31. Defibrinated blood.—White and black, female fox terrier, No. 385. January 29, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood since January 22. November 30, 1909. Dissection of the left carotid. Normal pulsations. Marked adhesions to the

vagus. Extirpation. Transplanted segment similar to the other parts of the carotid. *Microscopical examination.*—Section 265. No intima. Media: abnormally thin and composed of elastic fibers and amorphous substance. All muscle fibers have disappeared. Adventitia very much thickened.

Experiment 32. Defibrinated Blood.—Small, black and tan, male dog, No. 392. February 5, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood since January 22, 1909. *Microscopical examination.*—February 5, 1909. Section 212. Some muscle fiber nuclei uniformly stained and deformed. October 18, 1909. Dog died at the farm. *Macroscopical examination.*—Dissection of the artery. Transplanted segment of normal caliber. Slight adhesions to the vagus. Consistency of the wall harder. Its color is modified by the presence of yellow and hard patches. Anastomoses smooth and glistening. The patches are located between the media and the intima. The wall is thickened, but the lumen of the transplanted segment and of the anastomoses is not modified. *Microscopical examination.*—Section 253. Adventitia normal. Media deeply modified. In some parts it is very thin on account of the complete disappearance of the muscular fibers. It is composed only of the elastic framework and an amorphous substance. In some other parts, there are still some elongated cells between the elastic laminæ. The intima is exceedingly thickened. It is, in certain parts, five or six times thicker than the media and is composed of dense connective tissue. In the middle of the adventitia, the connective tissue has undergone calcareous degeneration.

Experiment 33. Defibrinated Blood.—Black, male dog, No. 402. February 11, 1909. Transplantation on the left carotid of a segment of carotid extirpated February 4, 1909. November 14, 1909. Dog in excellent condition. Dissection of the artery. Normal size, normal pulsations, no adhesions to the vagus. The location of the transplanted segment cannot be determined for the anastomoses are invisible. Resection and longitudinal incision of the artery. The lines of union are easily seen on the internal surface of the vessel. Anastomoses perfect. *Histological examination.*—Section 258. Intima a little thickened. Media: elastic framework normal. All the muscular fibers have disappeared. Adventitia very much thickened.

Experiment 34. Defibrinated Blood.—Brown dog, No. 408. February 17, 1909. Transplantation on the left carotid of a segment of carotid preserved for 48 hours in defibrinated blood and suprarenal. October 10, 1909. Dog killed in fighting other dogs. *Macroscopical examination.*—No evidence of the operation. No reaction of the connective tissue. No modification of caliber, color and consistency of the transplanted segment which cannot be located. Carotid artery is extirpated and longitudinally opened. The location of the segment is detected by a slight difference in the color of the intima which is a little yellowish. *Microscopical examination.*—Section 226. Adventitia normal. Media: elastic framework normal. No muscular fibers. Intima thickened and composed of dense connective tissue.

Experiment 35. Defibrinated Blood.—Male dog, No. 410. February 18, 1909. Transplantation on the left carotid of a segment of carotid preserved in defibrinated blood and thyroid since February 15, 1909. February 27, 1909. Opening of the neck. Circulation normal. Intense reaction of the connective tissue. June 5, 1909. Dog killed by accident. *Macroscopical examination.*—Very

marked adhesions of the transplanted segment to the vagus. Segment normal, although its caliber is slightly larger than that of the carotid. Anastomoses perfect. *Microscopical examination.*—Section 235. No intima. Media: normal elastic framework. No muscular fibers, although very few elongated cells are seen between the elastic laminæ. Adventitia very much thickened and composed of dense connective tissue. In several places the adventitia is thicker than the media and contains a great number of small vessels.

Experiment 36. Defibrinated Blood.—Black and tan bitch, No. 419. March 2, 1909. Transplantation on the left carotid of a segment of carotid extirpated on February 4, 1909. November 14, 1909. Dissection of the left carotid. The location of the transplanted segment can hardly be determined. The carotid has everywhere the same appearance and consistency. Resection of the artery. As soon as the circulation is stopped, the location of the segment becomes apparent, because it contracts less than the normal parts of the artery (Plate XLIII, Fig. 7). *Microscopical examination.*—Section 259. Intima a little thickened and containing a few elongated nuclei, assuming the appearance of muscle cells nuclei. Media: elastic frame-work normal. No muscular cells in the outer layers of the media. Cells with elongated nuclei in the middle and inner layers. Adventitia normal.

Experiment 37. Defibrinated Blood.—Yellow, male dog, No. 448. May 5, 1909. Transplantation on the right carotid of a segment of carotid extirpated on February 4, 1909. November 30, 1909. Dissection of the right carotid. No pulsations. Segment obliterated and transformed in a fibrous tract.

In four experiments, the vessels were preserved in vaselin.

Experiment 38. Vaselin.—Male dog, No. 450. May 6, 1909. Transplantation on the left carotid of a segment of carotid artery preserved in vaselin and in cold storage for 24 hours. August 11, 1909. Dog killed in a fight. *Macroscopical examination.*—Segment is obliterated by a clot of recent formation adherent to the wall at the level of its middle part. Anastomoses normal and free of clot. *Microscopical examination.*—Section 254. Intima slightly thickened. Media: elastic framework normal. Muscle fibers, nuclei normal. However, in a few places, between the two or three inner layers of the elastic fibers, they are missing. Adventitia normal.

Experiment 39. Vaselin.—Light yellow bitch, No. 454. May 12, 1909. Transplantation on the right carotid artery of a segment of carotid extirpated from a dog on May 5, 1909, and preserved in vaselin. November 30, 1909. Dissection of the carotid. No pulsations. Transplanted segment obliterated and atrophied. The ends of the carotid artery are united by a very small vessel. Its wall is very thin and its lumen obliterated by a thrombus.

Experiment 40. Vaselin.—Medium-sized dog, No. 458. May 19, 1909. Transplantation on the right carotid artery of a piece of artery preserved in vaselin since May 5, 1909. October 22, 1909. Dog in excellent health. Incision of the neck. Normal circulations. Carotid normal. Anastomoses almost invisible. Resection of the grafted segment. *Microscopical examination.*—Section 252. Adventitia normal. Media: elastic framework normal. Muscle fibers of the outer layers have disappeared. Muscle fibers of the middle and inner layers normal. Intima thickened, and infiltrated by muscle fibers. (Plate XLIII, Fig. 8).

Experiment 41. Vaselin.—White and black dog, No. 460. May 28, 1909. Transplantation on the right carotid of a segment of carotid extirpated on May 5, 1909, and preserved in vaselin. November 29, 1909. Dissection of the right carotid. Normal appearance, normal pulsations. Transplanted segment same caliber as carotid. Anastomoses are easily seen. Resection of carotid and transplanted segment. Intima smooth and glistening. Anastomoses perfect. *Microscopical examination.*—Section 264. Intima very much thickened and composed of connective tissue and of elongated cells which assume the appearance of muscle cells. Media thinner than normal. Almost all the muscle fibers have disappeared. It is composed of elastic fibers, of an amorphous substance, and of a very small number of muscle cells. Adventitia slightly thickened.

IV. RESULTS.

It must be known, from these experiments, whether an artery can be preserved outside of the body in a condition of latent life, and whether a preserved vessel can play safely the rôle of an artery. The results, therefore, will be examined from both standpoints, biological and surgical.

(*a*) *Biological Results.*—It is impossible to know directly whether or not a tissue which has been preserved for a few days or a few weeks in cold storage is living. There is no chemical reagent of life. The morphology of an artery gives little indication on its biological condition. A pig's carotid artery, which had been kept for six months in cold storage, had the same histological appearance as a fresh artery. There is no morphological difference between a living tissue and a dead tissue. What difference can be detected between the seed which is sterile and the seed which will produce a large tree? The only method of finding out the state of a tissue which is supposed to be in latent life is to replace it under normal physico-chemical conditions and to observe whether it will again manifest life. If two vascular segments which have been preserved in cold storage and assumed the same histological appearance are transplanted into a normal artery, and if after a few weeks one has retained its normal constitution while the other one has degenerated, it can be concluded that the first one was really in latent life while the second one was dead. However, the persistency of the elastic framework and of the connective tissue coats alone of the transplanted segment would not warrant the assumption that the vessel was still living at the time of the trans-

plantation. In such a case, the arterial wall might be possibly a mere outgrowth of the connective tissue from the host, the transplanted segment acting as a scaffold. But the presence of muscle fibers can demonstrate that the wall of the transplanted vessel is still present and is not merely formed by the tissues of the host.

The anatomical change undergone by dead vessels when grafted to a normal artery are shown by the experiments in which arterial segments killed by heat, formalin and glycerin were used. The dead vessels were examined just before being transplanted. The artery killed by formalin was absolutely normal. In the segments killed by heat and glycerin, the muscle cell nuclei were well stained but more or less deformed and retracted. As a whole, the vascular wall had kept an almost normal appearance. These vessels were transplanted into normal arteries and examined again a short time after the operation. In every case, a complete change had taken place. The segment, killed by formalin, spent eight days on the carotid of a dog. During this time, all the muscle fibers of the media disappeared (Fig. 1) and were replaced by amorphous material, while the elastic framework remained normal. The perivascular connective tissue of the host was very much thickened and was building a new wall around the dead structure. The artery killed by glycerin underwent similar changes. All the muscle fibers had disappeared eight days after the operation. Fourteen days after the transplantation, the media of the artery killed by heating was composed of stretched and broken elastic fibers, and of amorphous material, while the adventitia was thickened. These results are similar to those obtained previously by Levin and Larkin (7) in the transplantation of devitalized arterial segments.

These experiments show that a dead vessel undergoes, when transplanted, a rapid degeneration of all its muscle fibers (Fig. 1), while the elastic framework can remain normal. The change takes place in less than eight days. The host reacts against the dead vessel by forming around it a tube of dense connective tissue. A devitalized vessel acts as a foreign body, which can be progressively resorbed and replaced by the tissues of the host.

The arteries which were preserved by freezing behaved like dead

vessels. In Experiment 8, the transplanted segment was examined five days after the transplantation. All the muscle fibers had already disappeared and were replaced by amorphous material. The second experiment was also unsuccessful. These negative results do not mean that frozen arteries cannot be revived. It is well known, since the work of Ehrlich, that tumors, which have been frozen, can grow again normally. Other causes than freezing and especially non-suppurative infection may be responsible for the failure of my two experiments.

The results given by the arterial segments preserved by desiccation are very much better. Four experiments were performed. After having been in cold storage for a few hours in a sealed tube filled with calcium chloride, the arteries shrank and became brown and hard like a strand of catgut. In Experiment 6, the tube was then heated at 100° C. for ten minutes. Afterwards, the arteries were put in Locke's solution and regained their normal appearance. Their anatomical condition was practically normal. Nevertheless, they behaved differently after having been transplanted. In Experiment 4, all the muscle fibers, without exception, had disappeared seven days after the operation. The anatomical changes undergone by the vessel resembled very much those of vessels killed by formalin or glycerin. In Experiment 7, where the vessel had been dried, and in Experiment 6, where it had been dried and heated at 100° C., the results were different. The media was almost entirely composed of elastic fibers and amorphous material six months after the operation in the first case (Fig. 2), and nineteen days after the operation in the second case. Nevertheless, in the outer layers of the media, a few elongated nuclei, morphologically similar to muscle cell nuclei, could be detected. In Experiment 5, nineteen days after the operation, there were, in the outer layers of the media, a large number of normal muscle cell nuclei. It shows that some muscle cells had not been killed by the desiccation. This method may perhaps be improved and give better results. It is well known that in the drying of rotifers, a very slight difference of technique modifies widely the results. When a rotifer is dried on a glass plate, it cannot be brought back to life. If it is dried in sand, the resurrection is almost constant. The

rapidity of the desiccation plays doubtless an important rôle in the production of irreparable injuries in the architecture of the tissues of the cells. The fact that nineteen days after the transplantation of an artery which has been dried, many muscle fibers are still normal, shows that desiccation does not kill necessarily the tissues of the artery.

The results of the transplantation of vessels preserved in cold storage are divided in several classes, according to the medium in which the tissues were kept.

In three experiments, the arteries were placed in Locke's solution or in a 10/1000 solution of sodium chloride. In Experiment 10 the artery, kept for twenty-four hours in saline solution, was extirpated thirty-five days after the operation. The muscle fibers of the inner layers of the media had disappeared, but elsewhere they were normal (Fig. 3). The artery used in Experiment 11 was kept six days in saline solution. Forty-four days after the transplantation, the muscle fibers were still in excellent condition. In spite of the toxic action of the sodium ions for the tissues, an artery can be preserved for twenty-four hours and six days in physiological saline solution without being killed. In Experiment 12 a segment of artery was kept for nine days in Locke's solution, and then transplanted into a dog. The result was examined fourteen months after the operation. All the muscular fibers had degenerated.

In fifteen experiments the arteries were preserved in confined humid air. After a period varying from two days to eleven months, they were removed from their sealed tubes and transplanted. When they were examined before the transplantation, their appearance was generally found normal. Even after several months, the gross anatomy of the vessels was very little modified. The histological examination showed that they were often normal.

The results of the experiments are divided in four classes. The first class is composed of Experiments, 19, 20 and 26, in which the vessels were preserved from seven to eleven months outside of the body. Necrosis occurred soon after the transplantation. The second class consists of Experiments 14, 16, 18, 21 and 25 in which the muscle fibers degenerated completely, and were replaced by an

amorphous material (Fig. 5), while the elastic framework remained normal. Compensating changes took place in the adventitia and the intima. Therefore, these vessels behaved very much like vessels killed by heat, formalin or glycerin. The period of preservation of the vessels outside of the body had lasted from fourteen to seventy-two days. The examination of the transplanted vessel was performed from six days to six months after the operation. The third class is composed of Experiments 13, 15, 24 and 27. The arteries were partially killed during the period of preservation, which lasted from two days to twenty-one days. The results of the transplantation were examined from eight days to eighteen months after the operation. Some muscle fibers were still normal even after eighten months. In the fourth class, composed of Experiments 17, 22 and 23, the media was found to be normal. The arteries had been preserved for two days, seven days, and seventeen days outside of the body. The results of the transplantation were examined twenty-one days and fifteen days after the operation. The muscle fibers had remained normal (Fig. 6).

The marked differences observed in the results are due to several different causes. It is probable that the changes of temperature, the slight infection occurring during the period of preservation and after the transplantation caused many of the lesions found in the vessels. Several of the experiments were made when the temperature of the refrigerator was very inconstant and when the asepsis of the laboratory was doubtful. The preservation in confined humid air is not alone responsible for the observed degenerative changes of the arterial wall.

The preservation of arteries in serum or defibrinated blood was used in ten experiments. The period of preservation lasted from two to thirty-one days. The results were examined from fifteen days to nine months after the operation. In Experiment 28 the histological examination was not made. In Experiment 37 the transplanted vessel was resorbed. The results of the eight remaining experiments can be divided in two classes according to the conditions of the muscle fibers. In two experiments, 29 and 30, where the vessels were preserved for two days and four days, the muscle fibers remained normal. In the six other experiments

all the muscular fibers had disappeared. The elastic framework was normal and the muscular fibers were replaced by amorphous material. In the conditions of my experiments, serum or defibrinated blood acted as defective preservative media and their results were inferior to those of confined humid air.

The results of the four experiments of preservation of vessels in vaselin were very much better, although in Experiment 39, the artery became occluded and atrophied. The accident was due probably to a fault in the technique of the anastomoses and not to the method of preservation. In Experiments 38, 40 and 41, the vessels were kept in vaselin for twenty-four hours, fourteen days, and twenty-three days. In Experiment 38, the artery was examined and the specimen removed three months after the operation. A great many muscular fibers were normal. In the intima there were elongated nuclei which resembled muscle cells nuclei. The results of Experiment 40 were examined five months after the operation. A great many normal muscle cells were found in the media and in the intima. The muscle cells of the outer layers of the media had degenerated (Fig. 8). In Experiment 41, almost all the muscle fibers of the media had disappeared, six months after the operation. A few only were seen in the inner layers of the media. But the intima is thickened and contains many muscle fibers. In these experiments the preservation of the muscle fibers depends on the duration of the period of preservation outside of the body. It must also be noticed that the degenerated fibers are located in the outer part of the media, while they are in its inner part in the other methods, and also that they proliferated into the thickened intima.

All preservative media, salt solution, Locke's solution, confined humid air, defibrinated blood and vaselin permit the vessel to live in latent life under certain conditions, but vaselin appears to be the best medium.

(*b*) *Surgical Results.*—The practical value of a vessel preserved in a condition of latent life depends on its ability to act as a canal for the arterial blood. The only complication which interfered occasionally with the function of the transplanted vessel was thrombosis. The method used in the preservation of the vessel

has a marked influence on the occurrence of obliteration. When the arteries were killed by heat, formalin and glycerin, thrombosis occurred always. The results were a little better when the arteries were frozen or dried. In six operations, two positive results only were observed. In the experiments where the arteries were preserved in salt solution, Locke's solution or confined humid air, the results were very much better. If we eliminate three experiments in which the vessels were kept for more than six months in cold storage, we find ten positive results for fifteen operations. The vessels preserved in defibrinated blood gave eight positive results in ten experiments, and the vessels preserved in vaselin, three positive results for four experiments.

Negative results were observed after the transplantation of devitalized arteries. In every case, the segment was found after a few days, occluded by a thrombus. Levin and Larkin (7) also observed frequently the same accident. Nevertheless, thrombosis is by no means a necessary consequence of the graft of a dead artery. It is certain that the circulation can take place normally through an inert structure for some time at least. Once I performed the patching of the abdominal aorta of a dog with a piece of rubber, and the circulation went on normally. Guthrie (8) claims to have observed a normal circulation through a formalined vessel. Levin and Larkin found that ten days after an aortic graft, the circulation was still normal. The artery acts as a foreign body around which the organism builds a new wall of connective tissue (Fig. 1). On the intima of the dead vessels, the blood deposits a thin layer of fibrin, and it is possible that the dead structure is progressively resorbed and replaced by the connective tissue of the host. The devitalized artery is a resorbable foreign body. Nevertheless, it must be emphasized that it is a dangerous method. It can yield occasionally some good results. But immediate or secondary thrombosis occurs very much more often than in the case of transplantation of a living structure.

The results of the transplantation of frozen or dried vessels are better. Frozen vessels were transplanted twice and twice thrombosis occurred a short time after the operation. In four experiments of graft of dried vessels, thrombosis took place twice. The

two positive results were found nineteen days and six months after the operation. In Experiment 6, an artery which had been dried and then heated at 100° C. for twelve minutes was transplanted. Nineteen days after the operation, the neck was opened and the transplanted segment was found to have the same appearance, caliber and consistency as the carotid. The circulation was normal. As the muscular fibers of the media had degenerated, the vessel had adapted its wall to the pressure by increasing its adventitia which was two or three times thicker than the media. In Experiment 7, the result of the transplantation of a dried vessel was examined six months and eighteen days after the operation. The grafted segment assumed the same color, caliber and consistency as the other parts of the carotid. The morphologic and functional adaptation of the vessel was excellent. The media was weakened by the almost complete disappearance of the muscle fibers. But the elastic framework of the media was still normal, while a great many small elastic fibers had developed in the intima, which was very much thickened (Fig. 2). These experiments show that perfect functional results can be obtained by transplanting dried arteries. But the method is not safe, since thrombosis occurred twice in four experiments. It is possibly due to the technique I used. It would be necessary to repeat the experiments with a better method, and to try to preserve the dried vessels at a very low temperature, as Becquerel did with seeds. If tissues of mammals could be placed in potential life, it would be the ideal condition for the preservation of vessels outside of the body.

The results of the transplantation of arteries preserved in cold storage were examined from a few days to eighteen months after the operation. The proportion of positive results was 66 per cent. when the vessels were kept in Locke's solution and confined humid air, the experiments in which they were preserved outside of the body for more than six months not being counted. The proportion became 75 and 80 per cent. when the arteries were preserved in defibrinated blood or in vaselin.

The only complication which occurred was thrombosis. There were no aneurysms nor dilatation of the transplanted segment. In Experiment 19 a hemorrhage due to necrosis of the segment was

observed. The artery had been preserved for seven months in cold storage. The method cannot be expected to prevent the disintegration of a tissue after such a long time. Thrombosis is really the only complication to be feared. It is difficult to know its causes. Certainly, in several cases, thrombosis was not due to the method of preservation but merely to a fault in the technique of the suture, or to non-suppurative infection. It must be noticed that thrombosis occurred mainly during the period where the asepsis of the laboratory was doubtful. The percentage of positive results can certainly be increased. Most of the dogs were medium and small sized animals. By using larger vessels, for instance, vessels like the human humeral artery, better results could be obtained. Nevertheless, the method of preservation has certainly a marked influence. It seems that vaselin or defibrinated blood are the best media.

The function of the transplanted segment remained normal, even in the cases where the wall has undergone marked histological changes. The new artery adapted itself exactly to the vessel on which it was grafted. The union became so perfect that after a few months, it was difficult to find the location of the transplanted segment. In several experiments, the dissection of the carotid artery did not permit the locating of the graft (Figs. 4 and 7). It was necessary to open the vessel longitudinally in order to see the small linear scars or the faint change of color which were the only evidences of the operation. As soon as the vessel was empty of blood, the transplanted segment could be located easily. When the circulation stopped, the normal wall contracted itself, while the transplanted wall generally did not. The new segment appeared then as a slightly dilated portion of the vessel. In Experiment 16 a segment of carotid had been transplanted on a dog, after having been preserved in confined humid aid and in cold storage for twenty-two days. Six months after the operation, both carotid arteries were dissected. No evidence of the operation was seen. Both carotids had the same color, caliber and consistency. The transplanted segment had become absolutely identical to the normal parts of the carotid. Nevertheless, after the opening of the vessels, it became a little larger than the normal parts of the artery

and could be exactly located. The new arterial segment adapts itself, from a morphological standpoint, to the functionating artery.

In two experiments only the arterial wall underwent pathological modifications. Calcification and atheromatous patches, almost identical to the lesions observed in human arteriosclerosis, developed in its wall. But compensatory changes occurred and the caliber of the vessel was not appreciably modified.

The functions and the macroscopical appearance of the transplanted vessels did not depend at all on its histological architecture. For instance, in Experiment 18 (Fig. 5) the artery, five months after the transplantation, did not show any evidence of the operation. In Experiment 40 (Fig. 8) the artery, five months after the operation, was also normal. The only difference was that in Experiment 40 the wall of the transplanted segment contracted itself slightly after the stopping of the circulation, while in Experiment 18 it did not contract at all. Nevertheless, from a histological standpoint, the differences between these vessels were exceedingly marked. In Experiment 40, the wall was almost normal, although the muscle fibers of the outer layers of the media had disappeared. Compensatory change had taken place in the intima which was thickened and infiltrated by muscle fibers. In Experiment 18, the muscle fibers had been destroyed. The wall was composed only of an almost normal elastic framework and of connective tissue. But compensatory changes, such as thickening of the intima and of the adventitia had taken place, and the resistance and consequently the caliber of the vessel was not modified.

In many other cases the same modifications occurred. When the media was weakened by partial or complete degeneration of the muscular fibers, the wall increased its strength by thickening its intima, or its adventitia, or both media and adventitia. When the lesions of the media were more extensive in one point, the compensatory changes of the intima were marked at the same point. The compensatory changes consisted mainly of a sclerosis of the intima or of the adventitia. Very seldom, new elastic fibers developed in the intima. In the arteries preserved in vaselin, the muscle cells invaded the intima. These changes are adaptive changes which enable the weakened wall to resist the blood pres-

sure. It is the experimental demonstration of the theory advocated by Adami (9) in the development of arteriosclerosis. The thickening of the intima or of the adventitia was surely a compensatory change due to the weakening of the media. When the media was normal, no sclerosis of the other coats took place. The degree of sclerosis was proportional to the extent of the lesions of the media.

Not a single instance of arterial dilatation was observed. If the wall is composed of normal tissue, it reacts against the blood pressure by thickening itself. The function redintegrates quickly the weakened vessel. But its morphology is reëstablished only in the measure necessary to the function.

V. CONCLUSION.

When a segment of artery, killed by heat, formalin or glycerin is transplanted, it undergoes a rapid degeneration. Its muscle fibers disappear while the tissue of the host reacts by building a new wall of connective tissue. When the transplanted vessel has been preserved in a condition of latent life, no degeneration of the wall occurs, or the wall undergoes only partial degeneration. The muscle fibers can keep their normal appearance, even for a long time after the operation. It is, therefore, demonstrated that arteries can be preserved outside of the body in a condition of unmanifested actual life.

The best method of preservation consists of placing the vessels, immersed in vaselin, in an ice box, the temperature of which is slightly above the freezing point.

From a surgical standpoint, the transplantation of preserved vessels can be used with some safety. When the arteries were kept in defibrinated blood or vaselin and in cold storage, the proportion of positive results was 75 and 80 per cent., and this can probably be increased.

EXPLANATION OF PLATES.

PLATE XL.

FIG. 1. Formalined carotid artery. Eight days after transplantation. Experiment 1. Section 166. Hematoxylin-eosin.

FIG. 2. Carotid artery preserved by drying. Six months after transplantation. Experiment 7. Section 236. Weigert's elastic tissue stain.

PLATE XLI.

FIG. 3. Carotid artery preserved for 24 hours in salt solution and in cold storage. Thirty-five days after transplantation. Experiment 10. Section 5. Hematoxylin-eosin.

FIG. 4. Carotid artery preserved for 22 days in confined humid air and in cold storage. Six months after transplantation. Experiment 16.

PLATE XLII.

FIG. 5. Carotid artery preserved for 72 days in confined humid air and in cold storage. Five months after transplantation. Experiment 18. Section 122. Hematoxylin-eosin.

FIG. 6. Carotid artery preserved for 2 days in confined humid air and in cold storage. Fifteen days after transplantation. Experiment 23. Section 162. Hematoxylin-eosin.

PLATE XLIII.

FIG. 7. Carotid artery preserved for 26 days in defibrinated blood and cold storage. Eight months after transplantation. Experiment 36.

FIG. 8. Carotid artery preserved for 14 days in vaselin and in cold storage. Five months after transplantation. Experiment 40. Section 252. Hematoxylin-eosin.

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FIG. 1.

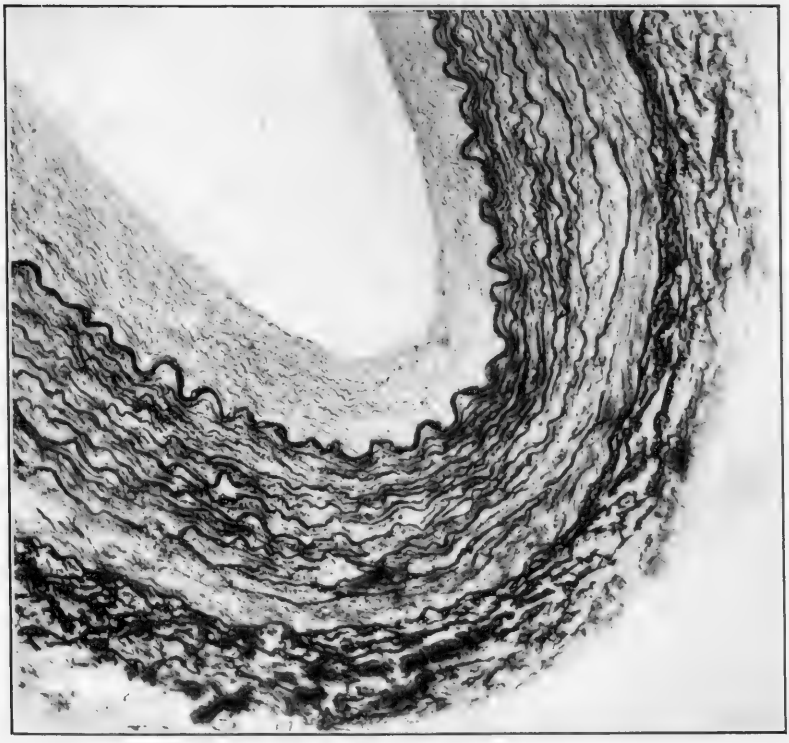

FIG. 2.

FIG. 3.

FIG. 4.

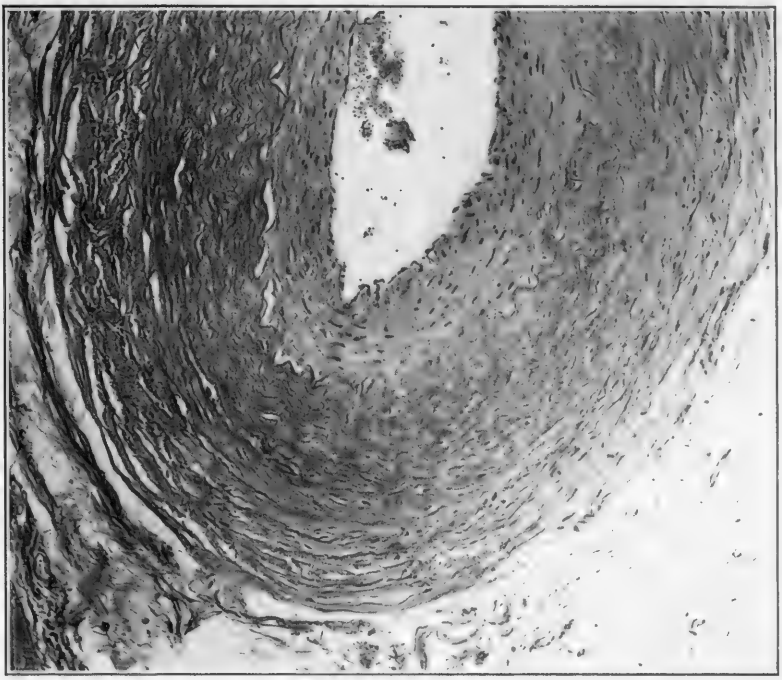

FIG. 5.

FIG. 6.

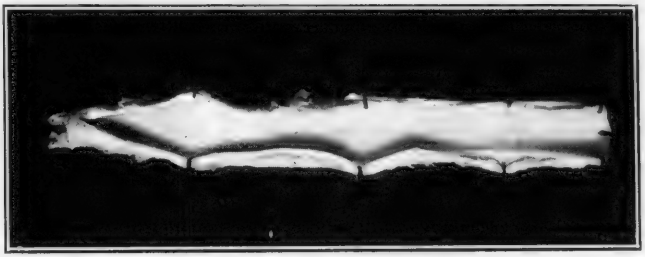

FIG. 7.

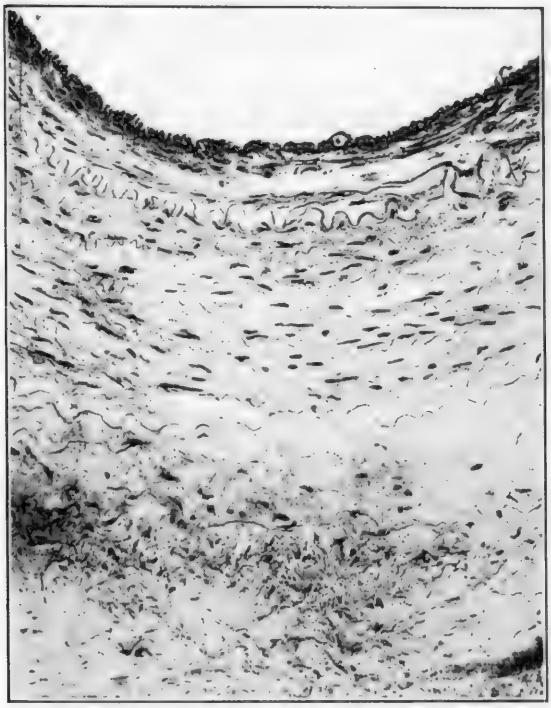

FIG. 8.

STUDIES IN EDEMA.

VII. The Influence of Nephrectomy and other Surgical Operations and of the Lesions produced by Uranium Nitrate upon Absorption from the Peritoneal Cavity.[1]

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In order to determine further the factors which influence absorption from the peritoneal cavity, we studied the absorption in nephrectomized animals and in animals injected with uranium nitrate, twenty and seventy-two hours after the injection.

All details regarding experimentation will be found in a previous communication (1).

The following table shows the principal figures to which we shall refer in this communication.

Absorption from the Peritoneal Cavity in Nephrectomized Animals.—In these experiments the kidneys were removed or the vessels were ligated through lumbar incisions. In the majority of the animals the peritoneum was not injured during the operation, but in a few cases it was slightly torn. We find by comparing the results in experiments in which the peritoneum was injured with the results in those in which we found the peritoneum intact, that such injury does not influence the absorption to any great extent. In some of our experiments the absorption was tested immediately after the operation; in the majority of our experiments we waited for eighteen to twenty hours after the operation before the absorption was tested. The results, however, were the same under both conditions. It also appeared that the results were the same in experiments in which we tied off the whole kidney stump, including vessels and ureter, and in experiments in which we tied off the

[1] Received for publication January 31, 1910.

ABSORPTION FROM THE PERITONEAL CAVITY.

	Blood before absorption.		Recovered fluid.						Blood.			Urine.	
	Sodium chloride per cent.	Δ	Amount in c.c.	Sodium chloride per cent.	Sodium chloride absorbed; in grm.	Sodium chloride absorbed pro c.c.	Δ	Refractive index	Sodium chloride per cent.	Δ	Dilution.	Amount in c.c.	Sodium chloride per cent.
Normal; 0.85 per cent. sodium chloride	.455	—0.60°	77	53	.588	.014	—0.593°	1.3353	.49	—0.586°	—.00133	11	.46
Normal; 0.85 per cent. sodium chloride; adrenalin intraperitoneally	—	—0.62	41	52	.811	.010	—0.593	1.3354	.47	—0.586	—.00065	41	.53
Normal; 0.85 per cent. sodium chloride; adrenalin intravenously	—	—	62	58	.72	.011	—0.593	—	.52	—0.593	—	11	.51
Normal; distilled water	—	—	44	40	—	—	—0.57	1.3361	.41	—0.583	—.00136	14	.32
Normal; distilled water; adrenalin intraperitoneally	—	—	21	36	—	—	—0.553	1.3359	.41	—0.58	—.0012	49	.40
Normal; 1.5 per cent. sodium chloride	—	—	116	59	1.1565	.2891	—0.634	1.3352	.55	—0.643	—.00026	8	.92
Normal; 1.5 per cent. sodium chloride; adrenalin intraperitoneally	—	—	81	62	1.2852	.0357	—0.654	1.3353	.51	—0.656	—.00036	30	.92
Nephrectomy	.466	—0.64	47	505	.796	.010	—0.59	1.3361	.487	—0.62	—.0015	0	—
Nephrectomy + adrenalin	—	—	43	49	.861	.0108	—	1.3364	.49	—	—.00105	0	—
1 day uranium nitrate	.495	—0.615	74	585	.606	.012	—0.59	—	.54	—0.60	—.0015	34	.41
1 day uranium nitrate + adrenalin	—	—	46	60	.794	.0095	—	—	.54	—	—.0005	64	.55
3 day uranium nitrate	.466	—0.675	77	595	.587	.013	—0.60	1.3364	.505	—0.63	—.0022	4	—
3 day uranium nitrate + adrenalin	—	—	64	57	.624	.011	—	1.3358	.50	—	—.0022	6	—
Etherization	—	—0.60	70	—	—	—	—	—	—	—0.59	—	20	—
Lugol's solution	—	—	71	—	—	—	—	—	—	—	—	13	—
Lumbar incision	—	—0.624	57	—	—	—	—	—	—	—	—.0015	18	—
Abdominal incision	—	—0.663	54	—	—	—	—	—	—	—0.64	—	—	—
Thoracic incision	—	—0.665	53	—	—	—	—0.609	—	—	—0.635	—.0009?	18	—
Peritonitis	—	—	133	—	—	—	—0.66	—	—	—0.71	—	—	—

ureter close to the pelvis of the kidney and left the renal circulation intact. In four instances in which the ureter was ligated we found that the results did not differ from those obtained when both the vessels and ureter were ligated.

In twenty-two experiments in which sodium chloride solution was injected into the peritoneal cavity of nephrectomized animals, an average of 47 cubic centimeters of fluid was recovered, while in nineteen experiments in which adrenalin was injected intraperitoneally in addition to the sodium chloride solution, an average of 43 cubic centimeters of fluid was recovered from the peritoneal cavity. We have found that in normal animals 77 cubic centimeters of fluid was recovered from the peritoneal cavity when 120 cubic centimeters of sodium chloride solution had been injected two and a half hours previously. We can, therefore, confirm the statements of Meltzer and Salant (2), and Achard and Caillard (3), that nephrectomy increases the absorption from the peritoneal cavity. In nephrectomized animals the influence of adrenalin in increasing the absorption from the peritoneal cavity has been markedly lessened.

von Koranyi (4) found the osmotic pressure of the blood in nephrectomized animals to be increased even after a period of four hours. In four nephrectomized animals we found the freezing point of the serum twenty-four hours after operation to be on the average —0.64°, a distinct increase over the normal osmotic pressure of the serum. In animals in which 0.85 per cent. sodium chloride solution had been injected into the peritoneal cavity, the mean of the lowering of the freezing point of the blood serum at the end of the experiment is —0.62°, and the mean of the lowering of the freezing point of the recovered peritoneal fluid is —0.59°.

Concerning the absorption of sodium chloride from the peritoneal cavity, we find that in the nephrectomized animals an average of 0.796 gram of sodium chloride was absorbed when 1.02 grams were injected, or .010 gram with every cubic centimeter of fluid. At the end of the experiment the sodium chloride percentage of the peritoneal fluid is 0.505 per cent.; in the blood serum the sodium chloride percentage is 0.487 per cent. In the nephrectomized animals which received injections of adrenalin, an average of

0.861 gram of sodium chloride was absorbed; thus .010 gram was absorbed with every cubic centimeter of fluid. The sodium chloride percentages in these latter experiments are practically the same as in the control experiments, namely, 0.49 per cent. in the peritoneal fluid and 0.49 per cent. in the blood serum.

It has been shown by Bence and Sarvonat (5) that in nephrectomized animals the blood becomes diluted a short time after the operation. We have found in four cases similar results and in four other cases the opposite results; in the latter, the blood became more concentrated. In the experiments of Bence and Sarvonat the increased amount of water in the blood was in many cases only relative, for they compared the loss of body weight and the change of the refractive index of the serum. In our experiments we made no comparison between body weight and refractive index of the serum, so that our results are expressed by the absolute figures.

After the injection of sodium chloride into the peritoneal cavity of nephrectomized rabbits, the blood became more dilute; the refractive index of the serum fell 0.0015. In nephrectomized animals into whose peritoneal cavities adrenalin was injected, the dilution of the blood was somewhat less, namely, .00105. The average refractive index of the recovered peritoneal fluid was 1.3361 in the control experiments and 1.3364 in the experiments in which adrenalin was injected.

It appears that nephrectomy has increased the relative absorption of sodium chloride from the peritoneal cavity. The remaining peritoneal fluid in normal animals, into whose peritoneal cavities we had injected 0.85 per cent. sodium chloride solution, contained 0.53 per cent., while the blood serum in such animals contained 0.49 per cent. of this salt; thus the sodium chloride percentage of the recovered peritoneal fluid is slightly lower in nephrectomized animals than in normal animals, while the sodium chloride percentage of the blood serum is approximately the same in both cases. In nephrectomized animals which were injected with adrenalin, the relative absorption of sodium chloride is also increased.

The absolute amount of sodium chloride absorbed in nephrec-

tomized animals is increased, inasmuch as only 0.588 gram was absorbed in the experiments with normal animals, as compared to 0.796 gram absorbed in nephectomized animals. The absolute amount of sodium chloride absorbed in nephrectomized animals which were injected with adrenalin was 0.861 gram as compared to 0.811 gram absorbed in normal animals injected with adrenalin. Thus in nephrectomized animals injected with adrenalin, a greater quantity of sodium chloride has been absorbed.

The dilution of the blood is slightly greater in the nephrectomized than in the normal animals. In nephrectomized animals we find slightly less fluid retained in the blood when adrenalin is injected than in control experiments, although the difference is not quite as marked as in the normal series. Here again adrenalin causes an additional elimination of fluid from the blood vessels.

Achard and Caillard (3) in studying the absorption in nephrectomized guinea pigs also found that the absorption of sodium sulphate or of sodium chloride of fluid was increased; and the movement of sodium chloride or urea into the peritoneal cavity appeared to be increased.

This increased absorption of fluid noted in nephrectomized animals is supposed by Meltzer and Salant (2) to be due to the increase in the osmotic pressure of the blood. They believe this increase in the osmotic pressure to be due to substances retained in the body as a result of the removal of the kidneys, and they assumed, therefore, that the increased absorption is a specific effect of the elimination of the kidney. The possibility existed that irritation of the peritoneum or some influence of the operative procedure, and not only the nephrectomy, might be a factor in increasing the absorption. We, therefore, determined the influence of irritation of the peritoneum and various other operative procedures on absorption.

As a mild irritant of the endothelial cells of the peritoneal cavity, we first used Lugol's solution. Two cubic centimeters of Lugol's solution were added to 120 cubic centimeters of sodium chloride solution, which were injected into the peritoneal cavity. We find in these experiments that the rate of absorption is not altered, inasmuch as we recovered from the peritoneal cavity an average of 71 cubic centimeters of fluid two and one-half hours after the injection.

It was necessary to determine the influence of etherization on absorption. We anesthetized six rabbits for fifteen minutes and on the following day tested the absorption in the usual manner. We recovered an average of 70 cubic centimeters of fluid and found the lowering of the freezing point of the blood serum to be —0.59°. We also determined the freezing point of the blood of animals which had been anesthetized twenty-four hours previously, but which had not been injected with sodium chloride solution, and found the average freezing point of the blood to be —0.60°, the same as we had found in normal animals. In one case we found the freezing point to be —0.64°, but since in the other four cases the freezing point was normal we must consider this as an exception. It appears that, when a rabbit has been deeply anesthetized twenty-four hours previously, the osmotic pressure of the blood is normal and the rate of absorption of fluid from the peritoneal cavity is not increased. It is true that Carlson, Greer and Becht (6) have shown that in dogs the osmotic pressure of the blood is increased during and for a short time after ether narcosis, but it appears from the experiments of Carlson and Luckhardt (7) that this increase in the osmotic pressure, which they suppose to be due to the ether dissolved in the blood, soon disappears and the osmotic pressure falls to an approximately normal level in a few hours.

In a third series of animals we operated in exactly the same manner as we would have done if we had intended to remove both kidneys. We made two lumbar incisions, separated the muscles until we brought into view the retroperitoneal tissues, then inserted a finger into the wound and irritated the peritoneum by friction on the external surface. We avoided as much as possible an injury or disturbance of the kidney. In these animals twenty-four hours after operation, we find the lowering of the freezing point of the blood increased, the average being —0.625°. When 120 cubic centimeters of 0.85 per cent. sodium chloride solution were injected into such animals, an average of 57 cubic centimeters of fluid was recovered from the peritoneal cavity. During this time 18 cubic centimeters of urine were secreted and the refractive index of the blood was diminished by 0.0015. Since in animals in which we

made a lumbar incision, the field of operation was in close proximity to the kidney and we might have slightly injured these organs, we carried out another series of experiments in which we made an abdominal incision in the linea alba, opened the peritoneal cavity and irritated the peritoneal surface. In such animals, on the day following the operation, the osmotic pressure of the blood is markedly increased; the lowering of the freezing point here shows an average of — 0.663°. In two experiments we find an average of 54 cubic centimeters of fluid recovered from the peritoneal cavity after the injection of 120 cubic centimeters of sodium chloride solution and we find the freezing point of the blood to be — 0.64°.

Might the increased absorption noted in these last two series be due to the irritation of the peritoneal endothelia or would a simple operation with a small degree of injury to the muscle and connective tissue, in which, however, the peritoneum was not injured, also cause increased absorption of fluid? An incision was made on both sides of the spinal column, well above the lower margin of the ribs; the aponeurosis and muscles were divided until the ribs and intercostal muscles were exposed. The muscles and skin were then sutured separately. About twenty-four hours after this operation the blood of such animals showed a lowering of the freezing point of — 0.665°; thus the osmotic pressure of the blood was increased. When 120 cubic centimeters of an 0.85 per cent. sodium chloride solution were injected into the peritoneal cavity of rabbits which had been so operated on, we recovered at the end of two and one-half hours an average of 53 cubic centimeters of fluid and found an average of 18 cubic centimeters of urine excreted. The recovered fluid showed an average freezing point of — 0.609°, and the blood serum, — 0.635°.

Reviewing these last few series of experiments, we find that neither etherization nor mild peritoneal irritation with Lugol's solution increases the osmotic pressure of the blood or the absorption of fluid from the peritoneal cavity. On the other hand, any operative procedure which involves more or less injury of the tissues increases the osmotic pressure of the blood and also the absorption of fluid from the peritoneal cavity.

From the results of the experiments in which a thoracic incision

was made, it is apparent that peritoneal irritation is not a necessary condition for increased absorption. Indeed, in three animals in which peritonitis developed following the abdominal incision, we recovered an average of 133 cubic centimeters of fluid two and one-half hours after the injection of 120 cubic centimeters of sodium chloride solution, In such animals we find the average of the freezing point of the blood serum to be —0.71° at the termination of the experiments, showing thus a markedly increased osmotic pressure of the blood.

In these latter experiments we have recovered from the peritoneal cavity more fluid than had been injected. We have to deal here not only with absorption from but also with transudation into the peritoneal cavity. The fluid recovered in these experiments was turbid and contained shreds of fibrin. In one case in which we determined the freezing point of the recovered fluid, it was —0.66°, showing a considerably higher osmotic pressure of the peritoneal fluid than we had noted in any other experiment.

The amounts of fluid absorbed in nephrectomized animals and in animals with operations not directly affecting the kidneys differed but slightly, so that the increased absorption noted in nephrectomized animals was, in the main, not a result of the removal of the kidneys but was due to an operation as such. In view of the fact that the rapidity of absorption in all operated animals was slightly less than in nephrectomized animals we cannot exclude the possibility that the removal of the kidneys has had some slight additional effect in increasing the absorption.

We find in all these experiments, in which animals were operated upon, that with increased osmotic pressure of the blood there is associated increased absorption of fluid and that in every case the osmotic pressure of the blood appears to be somewhat lowered by the absorption of fluid. In those cases in which the osmotic pressure of the blood is not increased—etherization and injection of Lugol's solution—we find no increased absorption of fluid. It thus appears that the absorption of fluid from the peritoneal cavity is mainly influenced by the osmotic pressure of the blood, a fact which agrees well with the results obtained in the experiments in which we tested the influence of adrenalin upon absorption.

We have found that the injection of adrenalin increases the absorption and that nephrectomy has the same action. Inasmuch as it is held by some authors that there exists an antagonism between the action of the adrenals and the kidneys, there is a possibility that removal of the adrenals may have an effect opposite to that of the injection of adrenalin and removal of the kidneys, in other words, that it may decrease the absorption from the peritoneal cavity.

In a series of five experiments we removed both adrenals. The animals so operated upon lived, as a rule, less than twenty-four hours. In only two animals did we determine the absorption of fluid twenty-four hours after operation; in the three others the absorption was determined about six hours after the operation, at which time the animals appeared to have completely recovered from the direct effect of the operation. The average amount of fluid recovered from the peritoneal cavity in such rabbits was 61 cubic centimeters. We find, therefore, that the rate of absorption is increased in rabbits from which the adrenals have been removed.

It is thus apparent that in such animals no specific influence of the loss of the adrenal glands is noted. The effect has been practically the same as that noted after any operation which does not affect the kidney, and it is probable that the increased absorption of fluid in animals in which the adrenals have been extirpated, is due to an increase of the osmotic pressure of the blood, such as has been noted after operations in which the kidneys and adrenals are not injured. This agrees well with our conception of the factors determining the absorption from the peritoneal cavity, inasmuch as adrenalin, extirpation of the kidneys and other operations, in the main, increase the absorption from the peritoneal cavity in direct proportion to the influence they exert on the osmotic pressure of the blood.

ABSORPTION IN ANIMALS INJECTED WITH URANIUM NITRATE ONE DAY PREVIOUSLY.

The rabbits used in this series of experiments had received a subcutaneous injection of five milligrams of uranium nitrate about twenty-four hours previous to the time at which the absorption was

studied. At this stage of the uranium nitrate poisoning the animal appears perfectly well and the only symptom manifest is an increased secretion of urine.

When 120 cubic centimeters of 0.85 per cent. sodium chloride solution were injected into the peritoneal cavity of such rabbits, we recovered, at the end of two and one-half hours, an average of 74 cubic centimeters of fluid. When in addition to the sodium chloride solution adrenalin was injected intraperitoneally, an average of 46 cubic centimeters of fluid was recovered. Here the adrenalin increased the absorption as markedly as it did in the normal animals. The conditions present in the rabbit one day after the injection of uranium nitrate do not seem to have influenced the absorption of fluid.

The sodium chloride percentage of the recovered peritoneal fluid in the control[2] animals in this series was .58 per cent, and, therefore, considerably higher than in normal animals, whereas the total amount of sodium chloride absorbed was 0.606 gram. In the adrenalin experiments the sodium chloride percentage of the recovered fluid was .60 per cent., rather higher than in the controls; the total amount of sodium chloride absorbed was 0.794 gram. With every cubic centimeter of fluid leaving the peritoneal cavity, .012 gram of sodium chloride was absorbed in the control experiments and 0.0095 gram in the adrenalin experiments.

It appears from the figures of the amounts of sodium chloride absorbed from the peritoneal cavity that more sodium chloride was absorbed in animals one day after the injection of uranium nitrate than in normal animals, in spite of the fact that the amount of fluid absorbed in these former animals was only a few cubic centimeters more than in the normal animals, while the sodium chloride percentage in the remaining peritoneal fluid was considerably greater in the former. This discrepancy is due to the fact that the sodium chloride percentages in each series were estimated in only five or six individual experiments. In this latter small series the average amount of fluid recovered in normal animals was 80 cubic centimeters,

[2] By "control-animals" we mean animals injected with uranium nitrate, but not with adrenalin or another additional substance. In control as well as in the other animals the absorption of solutions from the peritoneal cavity was determined.

therefore slightly above the average attained in the larger series in which all experiments were included. For the uranium nitrate series the average amount of fluid recovered in the experiments in which simultaneously sodium chloride determinations were made, was 71 cubic centimeters, therefore slightly less than the average obtained in the whole series of experiments. Thus in these smaller series of experiments, in which sodium chloride determinations had been made, the variations, although slight, followed opposite directions, so that the difference between the amounts of fluid absorbed is larger than in the more complete series of experiments. If we readjust the figures of the amounts of sodium chloride absorbed in accordance with the figures of the absolute amounts of fluid absorbed in the larger series of experiments, we will find that actually a little less sodium chloride is absorbed in the one day uranium nitrate animals than in normal animals. With this correction we find that about .612 gram of sodium chloride is absorbed in normal animals and .587 gram in animals which had received an injection of uranium nitrate. In the animals in which adrenalin was injected, we find less sodium chloride absorbed in experiments carried out with uranium nitrate animals than in the case of normal animals.

The lowering of the freezing point of the blood serum in rabbits one day after the injection of uranium nitrate is slightly increased; we have found this lowering to be approximately —0.615°. After absorption of sodium chloride solution, the freezing point of the blood rose to the level noted in the serum of normal animals, namely, —0.60°, but it did not rise to the level of the freezing point in normal animals in which absorption of a sodium chloride solution had taken place. The lowering of the freezing point of the recovered peritoneal fluid in control uranium nitrate animals is —0.59°, slightly less than in the serum.

One day after the injection of uranium nitrate, but before the intraperitoneal injection of sodium chloride solution, the sodium chloride content of the serum in rabbits was 0.495 per cent.; it was, therefore, increased over the normal sodium chloride content, which we found to be 0.455 per cent. on the average. This increase may perhaps be due to the increased diuresis, which, in the case of uranium nitrate animals, is accompanied by a decreased sodium chlo-

ride elimination, and we may thus have a relative retention of sodium chloride in the blood. It appears, however, more probable that the increased sodium chloride content of the blood is the primary condition and that the diminished sodium chloride elimination is due to this retention. After the absorption of a sodium chloride solution the sodium chloride content of the blood serum was 0.54 per cent. in animals which had been injected with uranium nitrate. The same percentage was found in animals which received additional injections of adrenalin. We find an increased sodium chloride percentage in the blood in spite of the increased diuresis and in spite of the fact that absolutely more sodium chloride is excreted in both these cases. This increased percentage of sodium chloride in the blood after absorption of a sodium chloride solution corresponds to the increased amount of sodium chloride noted in the blood before the injection of fluid into the peritoneal cavity.

In all the animals injected with uranium nitrate one day previously, the refractive index of the blood shows a reduction after the absorption of fluid. In control animals treated with uranium nitrate it is reduced by .0015 and in those which in addition received injections of adrenalin, by .0005. In the control uranium nitrate animals the dilution of the blood is slightly greater than in normal animals and we find that adrenalin diminished markedly the dilution of the blood here, as well as in normal animals.

As stated above, the animals injected with uranium nitrate one day previously secreted large quantities of urine, an average of 34 cubic centimeters of urine being secreted which contained .41 per cent. of sodium chloride, while in the animals which, in addition to the uranium nitrate received injections of adrenalin, an average of 64 cubic centimeters of urine was secreted, containing .55 per cent. of sodium chloride.

Therefore, we find that in animals which have been injected with uranium nitrate one day previously the rate of absorption of fluid from the peritoneal cavity is the same as in normal animals, and that adrenalin increases the absorption in such animals in a similar measure, as it does in normal animals. The relative absorption of sodium chloride from the peritoneal cavity is diminished in experiments with animals one day after the injection of uranium nitrate,

both with and without the injection of adrenalin, as shown by the high sodium chloride percentage of the remaining fluid. The diminished relative absorption of sodium chloride stands in relation to the high sodium chloride percentage in the blood serum. In this case the movement of sodium chloride appears, therefore, to have been regulated in conformity with the laws of diffusion. The absolute amount of sodium chloride absorbed is also diminished in animals injected with uranium nitrate one day previously. The fluid content of the blood is somewhat greater in these experiments than in experiments with normal animals, in spite of the markedly increased diuresis. This increased retention of fluid in the blood vessels may be due to the increased osmotic pressure of the blood. The influence of adrenalin causing fluid to pass out of the vessels is quite evident in this case, and the dilution of the blood following the injection of adrenalin is even slightly less in experiments with one day uranium nitrate animals than in experiments with normal animals.

As we saw in animals which were injected with uranium nitrate one day previously, adrenalin caused simultaneously increased absorption of fluid, increased diuresis with increased elimination of sodium chloride and lessened retention of fluid in the blood vessels in a similar manner as in normal animals. It appears that a correlation exists between these various phenomena caused by the injection of adrenalin.

ABSORPTION IN ANIMALS INJECTED WITH URANIUM NITRATE THREE DAYS PREVIOUSLY.

The rabbits used in these experiments were given an injection of five milligrams of uranium nitrate three days previous to the absorption experiments. The injection of this quantity of uranium nitrate is sufficient in most cases to produce renal insufficiency at the end of three days, so that little or no urine is secreted. The rabbits are not in a moribund condition and it is rare that edema is present at this stage.

In such uranium nitrate animals, after an intraperitoneal injection of 120 cubic centimeters of 0.85 per cent. sodium chloride solution, we recovered 77 cubic centimeters of peritoneal fluid at

the end of two and one-half hours. When, in addition to the sodium chloride solution, adrenalin was injected intraperitoneally, we recovered on the average 64 cubic centimeters of fluid. The rate of absorption of fluid from the peritoneal cavity was not changed by the anuria or the associated conditions produced by the injection of uranium nitrate. Under these conditions adrenalin, however, seems to have lost to a considerable extent its power to increase the absorption of fluid.

Richter (8) has shown that the lowering of the freezing point of the blood serum is increased in animals in which a nephritis has developed as a result of the injection of uranium nitrate. In four experiments we have found the average freezing point of the blood to be about —0.675° under such conditions. In the three day uranium nitrate animals which had received an injection of 120 cubic centimeters of 0.85 per cent. sodium chloride solution intraperitoneally, the average freezing point of the blood serum was —0.63°, while that of the peritoneal fluid was —0.60° at the end of the experiment. We found, therefore, the osmotic pressure of the blood serum increased in rabbits three days after the injection of uranium nitrate, and we also found that absorption of the injected sodium chloride solution caused a lowering of the osmotic pressure of the blood in a similar manner as in normal animals; however, the osmotic pressure did not fall to normal but remained still slightly higher at the end of the experiment.

The sodium chloride percentage of the blood serum has been found by some observers to be decreased, by others to be increased, in uranium nitrate nephritis. We have found the sodium chloride percentage in the blood serum in rabbits three days after the injection of uranium nitrate to be .466 per cent., which represents a very slight increase over the normal percentage.

In uranium nitrate animals into whose peritoneal cavities 120 cubic centimeters of 0.85 per cent. sodium chloride solution were injected, the sodium chloride content in the blood serum was 0.50 per cent., and in the peritoneal fluid, 0.0595 per cent. at the conclusion of the absorption experiments. When, in addition, adrenalin was injected intraperitoneally, the sodium chloride percentage in the blood serum was the same as in the uranium nitrate control

experiments, namely, 0.50 per cent.; the percentage in the peritoneal fluid was 0.57 per cent.

In the uranium nitrate control experiments, an average of 0.587 gram of sodium chloride was absorbed from the peritoneal cavity of each rabbit. Here, as in some previous experiments, the sodium chloride determinations had been made in a limited number of experiments and the estimate of the absolute amount of sodium chloride absorbed from the peritoneal cavity was based on the figures of the fluid recovered from the peritoneal cavity in those particular cases. If, however, these estimations had been calculated on the basis of the average of fluid absorbed in all experiments, the figure of the amount of sodium chloride absorbed from the peritoneal cavity would have been 0.574 gram, as compared with 0.612 gram in normal animals. In the adrenalin experiments an average of 0.624 gram of sodium chloride was absorbed from the peritoneal cavity. Adrenalin increases, therefore, the absorption of sodium chloride from the peritoneal cavity even in animals injected with uranium nitrate three days previously. If we compare, however, the absorption in normal animals and in these uranium nitrate animals, we find that both with and without the injection of adrenalin the relative absorption of sodium chloride and the absolute amounts of sodium chloride absorbed are reduced under the influence of uranium nitrate. It is not possible to explain the decreased sodium chloride absorption in animals three days after the injection of uranium nitrate on the basis of an increased sodium chloride percentage of the blood serum, as we did in the experiments with rabbits one day after the injection of uranium nitrate, inasmuch as three days after the injection of uranium nitrate the sodium chloride content of the blood was not found to be increased.

Bence (9) has shown that in the early stages of the uranium nitrate nephritis in rabbits the blood becomes more diluted, its refractive index being lowered; but later on, according to this author, the dilution of the blood gradually disappears and at one time, namely, towards the end of the period of increased diuresis, the blood even becomes slightly more concentrated. Afterwards the blood again becomes more diluted simultaneously with the appearance of edema. In four rabbits we determined the refractive index of the

blood before the injection of uranium nitrate and also three days after the injection; in one the refractive index was unchanged, in the other three there was considerable dilution of the blood. It appears, therefore, that the period at which we made our absorption experiments was near the beginning of the period of anuria when edema had not as yet developed.

The blood in animals three days after the injection of uranium nitrate, into whose peritoneal cavity sodium chloride solution was injected, showed an average decrease of the refractive index of .0022. The same average decrease of the refractive index was noted in the rabbits poisoned with uranium nitrate three days previously which received injections of adrenalin during the time of absorption of the sodium chloride solution. The average refractive index of the recovered peritoneal fluid was 1.3364 in experiments in which adrenalin was not injected, and 1.3358 in experiments in which it was injected.

In these experiments the blood has, therefore, the tendency to retain fluid even when no solution has been injected into the peritoneal cavity. When, however, such an injection has been made, this tendency toward retention of fluid in the blood becomes still more marked, inasmuch as the fluid content of the blood has appeared to be greater in experiments with animals three days after the injection of uranium nitrate than in any other series of experiments. This occurred in spite of the fact that the amount of fluid absorbed from the peritoneal cavity was not increased, so that the increased amount of water in the blood must in part have been drawn from the tissues.

The anuria and any other conditions which may be present in rabbits three days after the injection of uranium nitrate do not change the rate of absorption of fluid from the peritoneal cavity. It is very probable that the effect of the increase in the osmotic pressure of the blood is neutralized by some other condition. The influence of adrenalin in increasing the rate of absorption is, however, distinctly less marked and has almost disappeared in these animals. The relative absorption of sodium chloride from the peritoneal cavity is delayed in these experiments and the percentage of the sodium chloride in the recovered peritoneal fluid is, there-

fore, slightly higher in animals three days after the injection of uranium nitrate than in normal animals. The absolute amount of sodium chloride absorbed is also less. The injection of adrenalin increases slightly the relative as well as the absolute absorption of sodium chloride. As we stated above, the dilution of the blood after the injection of sodium chloride solution is greater in animals which were injected with uranium nitrate than in normal animals and adrenalin is no longer able to diminish the amount of fluid retained in the blood after administration of uranium nitrate.

We notice, therefore, that three days after an injection of uranium nitrate adrenalin is able to increase only slightly the absorption of fluid from the peritoneal cavity, that it does not exert any influence on the secretion of urine and that it does not cause more fluid to pass out of the blood vessels. It thus appears that there is a distinct relation between these three phenomena.

Achard and Caillard (3) found in guinea pigs which had been injected with uranium nitrate some days previously that less sodium sulphate was absorbed from the peritoneal cavity; when, however, the uranium nitrate was injected intraperitoneally simultaneously with the sodium sulphate solution, the sodium sulphate was absorbed more rapidly.

DISCUSSION.

We find in rabbits both one and three days after the injection of uranium nitrate that the osmotic pressure of the blood is increased, slightly in the former case and quite markedly in the latter, and yet the absorption from the peritoneal cavity is not increased in either of these series of experiments. We also note in experiments with animals in which peritonitis had developed increased osmotic pressure of the blood and yet decreased absorption. In these experiments some new and complicating factor comes into play, the character of which we cannot determine at the present time. It has been held by Richter (8) and Heinecke and Meyerstein (10) that uranium nitrate affects the endothelial cells of the blood vessels. If we accept provisionally this explanation of the action of uranium nitrate, we may assume that under its influence alterations in the blood vessels and perhaps also in the endothelial lining of the peritoneal cavity occur which produce changes in the

permeability of the membranes separating the peritoneal cavity from the blood. In a similar manner peritonitis may influence the endothelium of the peritoneum.

That there exists no direct relation between diuresis and absorption becomes quite evident in the nephrectomized animals in which the absorption is quite markedly increased, while in animals which were injected with uranium nitrate one day previously and which show a marked increase in diuresis, the absorption is in no way increased over that noted in normal animals. While no direct relation can be said to exist between increased diuresis and increased absorption yet in certain cases we find these two conditions associated. Thus in all the animals in which an operation, which did not directly affect the kidney was performed, as well as in normal animals injected with adrenalin intraperitoneally, we find the rate of absorption increased and also a slight increase in diuresis. After an operation the increased absorption of fluid from the peritoneal cavity is in all probability the primary condition and the increased diuresis is secondary.

If we summarize the influence of adrenalin on absorption from the peritoneal cavity under the conditions which we have considered in this paper, we find in all the series that the absolute amount of sodium chloride absorbed is increased. The relative amount of sodium chloride absorbed in experiments in which adrenalin was injected is slightly increased in nephrectomized animals and in animals injected with uranium nitrate three days previously. In animals, however, which have received an injection of uranium nitrate one day previously the relative amount of sodium chloride absorbed is diminished. Thus although adrenalin uniformly increases the absolute amount of sodium chloride absorbed, its influence on the relative absorption of sodium chloride varies.

In all the series of experiments reported in this paper we find the sodium chloride content of the blood to be greater at the end than at the beginning of the absorption. In the nephrectomized animals the sodium chloride percentage in the blood before the intraperitoneal injection of fluid is the same as in animals injected with uranium nitrate three days previously; but after the absorption of the sodium chloride solution the percentage is greater in the latter

(uranium nitrate) animals. After the absorption of fluid from the peritoneal cavity the dilution of the blood is also greater in animals three days after the injection of uranium nitrate than in the nephrectomized animals, so that the amount of sodium chloride retained in the blood in the former animals is even greater than appears from the percentage. From these considerations it follows that since in nephrectomized animals none of the sodium chloride can leave the body through the kidneys—as it does in small amounts in the three-day uranium nitrate animals or in quite large quantities in the one day uranium nitrate animals—a very large quantity of sodium chloride must be retained in the tissues of nephrectomized animals.

It may be that the decreased sodium chloride percentage in both the blood and the recovered peritoneal fluid of the nephrectomized animals is due to a change in the tissues of the body, as a result of which the absorptive power of the tissues for sodium chloride is increased.

The main difference between nephrectomized and normal animals, on the one hand, and animals injected with uranium nitrate, on the other, is that in the former the sodium chloride is to a great extent removed from the blood or peritoneal fluid either by elimination through the kidneys or by passing into the tissues, while in the latter condition a much smaller quantity of sodium chloride accumulates in the tissues. This lessening of the accumulation of sodium chloride in the tissues under the influence of uranium nitrate may be due either to decreased permeability of the vessels and peritoneal endothelium for sodium chloride or to a diminished capability of the tissues to bind the sodium chloride.

The amount of fluid retained in the blood after the intraperitoneal injection of fluid is the same in nephrectomized animals, and in animals injected with uranium nitrate one day previously. In animals, however, injected with uranium nitrate three days previously, the amount of fluid retained in the blood is considerably greater. The higher osmotic pressure of the blood in these latter animals may be a contributing factor in causing the greater dilution of the blood. That this is, however, not the only cause becomes evident when we consider the influence of the injection of adrenalin

in this condition; we note that here adrenalin does not diminish the dilution of the blood. It is possible that in agreement with the views of Richter (8), Heinecke and Meyerstein (10) and Schlayer, Hedinger and Takayasu (11), the reactions of the blood vessels are changed under the influence of uranium nitrate and that, therefore, after the administration of uranium nitrate, adrenalin is no longer able to cause more fluid to pass out of the blood vessels.

In nephrectomized animals larger amounts of osmotically active substances other than sodium chloride must pass into the peritoneal cavity than in normal animals; this follows from the fact that the freezing point of the recovered peritoneal fluid is approximately the same in both series of experiments notwithstanding the diminution in the sodium chloride percentage of the recovered peritoneal fluid in nephrectomized animals.

One day after the injection of uranium nitrate the freezing point of the peritoneal fluid differs very little from that noted in normal animals, but its sodium chloride content is considerably greater; the quantity of substances other than sodium chloride which might increase the osmotic pressure, and which have passed into the peritoneal cavity from the surrounding tissues and fluids, must, therefore, be smaller. Likewise three days after the injection of uranium nitrate the freezing point of the peritoneal fluid is very nearly the same as in normal animals, but the sodium chloride percentage is higher; consequently in these experiments again a smaller quantity of osmotically active substances other than sodium chloride must have passed into the peritoneal cavity.

In all the experiments reported in this paper we note that the osmotic pressure of the blood is slightly lower at the end than at the beginning of the experiments, a condition which we also noted in our previous paper, and, in view of the fact that the solution injected into the peritoneal cavity had a slightly lower osmotic pressure than the normal serum, this lowering of the osmotic pressure of the blood may easily be understood.

Throughout these experiments we find the osmotic pressure of the recovered peritoneal fluid to be less than that of the blood serum, while in experiments with normal animals into whose peritoneal cavity we had injected 0.85 per cent. sodium chloride

solution the reverse condition was found. Georgopulos (12) who studied the relative osmotic pressure of the edematous fluid and of the blood in animals at various stages of uranium nitrate nephritis found the osmotic pressure of the ascitic fluid to be the same as or less than that of the blood serum. On the other hand, Richter (8), who also studied the osmotic pressure of the ascitic fluid and the blood in animals injected with uranium nitrate, found the osmotic pressure of the ascitic fluid greater than that of the blood. Richter's determinations were made at a later stage of the intoxication than were ours and since we examined our rabbits at one period only—a period when edema had not yet appeared—our conclusions do not contradict those of Richter.

The absence of renal secretion may be a factor in causing this difference in the distribution of the osmotically active substances in the blood and peritoneal fluid. That there are, however, other factors present which influence this condition becomes apparent if we take into consideration the fact that, notwithstanding the increase in diuresis one day after the injection of uranium nitrate, the osmotic pressure of the blood in such animals is greater than that of the peritoneal fluid.

In both nephrectomized animals as well as in animals injected with uranium nitrate three days previously, the albumin content of the recovered peritoneal fluid is slightly higher than in normal animals.

Observations on the dilution of the blood and the occurrence of edema in nephrectomized and in uranium nitrate rabbits led Bence (9) to the conclusion that the conditions leading to the production of edema in both these animals were essentially the same; from our experiments it appears, however, that there exist marked differences between nephrectomized rabbits and rabbits with uranium nitrate poisoning. Such differences concern the absorption of fluid from the peritoneal cavity, the distribution of sodium chloride and other osmotically active substances in the blood and in the peritoneal cavity, and also the retention of fluid in the blood. These are factors of fundamental importance in the production of edema and the differences between nephrectomized animals and animals treated with uranium nitrate, which we have established; let us

expect that corresponding differences exist in regard to the readiness with which edema appears in these two classes of animals.

CONCLUSIONS.[3]

1. In the experiments recorded in this paper the influence of the osmotic pressure of the blood upon absorption of fluid from the peritoneal cavity becomes apparent. Nephrectomy, removal of the adrenals, and other operations increase the osmotic pressure of the blood and increase the absorption of fluid from the peritoneal cavity. On the other hand, ether narcosis, at the period at which we tested its influence, causes neither an increase of osmotic pressure of the blood nor an increase in the absorption of fluid from the peritoneal cavity.

2. The increased osmotic pressure and increased absorption of fluid in nephrectomized animals is to a great extent not a specific effect of the removal of the kidneys, but approximately the same conditions can be observed after incisions of the skin and muscles.

3. After poisoning with uranium nitrate and in cases of peritonitis, complicating factors come into play, and under such conditions the absorption from the peritoneal cavity is not increased, notwithstanding the higher osmotic pressure of the blood.

4. In conditions in which the osmotic pressure of the blood is very high before the injection of sodium chloride solution into the peritoneal cavity (nephrectomized rabbits or rabbits injected with uranium nitrate three days previously), adrenalin causes no increase, or only a very slight one, in the absorption of peritoneal fluid. On the other hand, one day after the injection of uranium nitrate the osmotic pressure of the blood is only slightly increased before the injection of the sodium chloride solution into the peritoneal cavity, and here adrenalin causes a marked increase in absorption of fluid from the peritoneal cavity.

5. In animals injected with uranium nitrate the retention of sodium chloride and other osmotically active substances in the blood is not entirely due to interference with the functions of the kidney.

[3] We did not include in this paper the records of the individual experiments in order to limit the size of this communication. It, may, however, be stated that the results communicated in this paper are based on 199 experiments carried out in an identical number of rabbits.

This retention may be explained either by an inability of the tissues to bind the sodium chloride and other osmotically active substances or to a diminished permeability of the blood vessels for such substances.

6. While in nephrectomized animals the elimination of sodium chloride from the peritoneal cavity and also from the blood is increased, in animals injected with uranium nitrate such an elimination is diminished. This increase in the sodium chloride content of the peritoneal fluid in animals treated with uranium nitrate is accompanied by a decrease in the diffusion of other osmotically active substances into the peritoneal cavity.

7. While in nephrectomized animals and in animals injected with uranium nitrate one day previously, adrenalin causes a diminution of the fluid retained in the blood-vessels similar to the diminution noted in normal animals, adrenalin no longer exerts such an effect at a later stage of the uranium nitrate poisoning. At this period after the administration of uranium nitrate, the retention of fluid in the blood vessels is apparently equal in experiments with and without the injection of adrenalin, and following the absorption of fluid from the peritoneal cavity, the retention of fluid in the blood vessels in the uranium nitrate animals is increased comparatively to a greater extent than in normal animals.

8. Our experiments show a marked difference in the distribution of fluid and of osmotically active substances in nephrectomized animals and in animals injected with uranium nitrate. This difference may explain the much greater liability to the development of edema in animals injected with uranium nitrate.

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STUDIES IN EDEMA.

VIII. The Influence of Caffeine on Absorption from the Peritoneal Cavity and the Influence of Diuresis on Edema.[1]

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From the results of some earlier experiments (1, 2) it appears that the action of adrenalin in increasing the rate of absorption from the peritoneal cavity is due, not to the increased diuresis caused by this drug, but to an increase of the osmotic pressure of the blood. We have also shown in nephrectomized animals, and in animals upon which an operation had been performed, not directly affecting the kidneys, that changes in the osmotic pressure of the blood will account for the increased rate of absorption under such conditions. Furthermore, we have studied the influence of these various substances and conditions upon the distribution of sodium chloride and other osmotically active substances in the blood and peritoneal fluid.

We, therefore, determined to test the validity of our former conclusions by an investigation on the influence of diuretics on absorption from the peritoneal cavity. Through such experiments we hoped to learn definitely whether diuresis stands in direct relation to absorption from the peritoneal cavity. We injected 120 cubic centimeters of 0.85 per cent. sodium chloride solution into the peritoneal cavity of rabbits. Afterwards four injections of caffeine sodium benzoate were given, separated by periods of one-half hour; 0.25 gram caffeine were given with each injection. After two and a half hours the rabbits were killed and the fluid in the peritoneal cavity was collected. During the experiment the urine also was collected. We determined the freezing point and sodium

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chloride content of the blood serum, which was obtained through defibrination and of the recovered peritoneal fluid, the sodium chloride content of the urine, the refractive index of the blood serum before and after the intraperitoneal injection of the sodium chloride solution and the refractive index of the peritoneal fluid.

In other series of experiments we studied the influence of caffeine on absorption in nephrectomized animals, in animals injected with uranium nitrate, and in animals in which, instead of nephrectomy, another operation, which did not directly affect the kidneys, had been performed.

In one series of experiments we injected, instead of the sodium chloride solution, distilled water into the peritoneal cavity of the rabbits, and again determined the influence of caffeine on absorption.

In some of the experiments with normal animals, we injected the caffeine subcutaneously, while in others the caffeine was injected intraperitoneally; the effect of caffeine upon absorption was not influenced by the method of administering the drug, as we recovered approximately the same quantities of fluid from the peritoneal cavity in each series of experiments. Therefore, in the following reports we have made no distinction between experiments in which caffeine was injected intraperitoneally and others in which it was injected subcutaneously.

THE INFLUENCE OF CAFFEINE ON ABSORPTION IN NORMAL ANIMALS.

When caffeine was injected in addition to the sodium chloride solution, we recovered from the peritoneal cavity an average of 82 cubic centimeters of fluid and collected during the experiments an amount of urine averaging 67 cubic centimeters. Caffeine has increased the diuresis considerably more than adrenalin, and notwithstanding this increase in diuresis the amount of fluid absorbed from the peritoneal cavity is slightly less than in experiments with normal control animals which have received neither adrenalin nor caffeine.

Both the relative and the absolute amount of sodium chloride absorbed from the peritoneal cavity have been increased by the injection of caffeine in normal animals. The blood serum of animals injected with caffeine in addition to the sodium chloride

solution contains 0.46 per cent. of sodium chloride, while the serum of animals which had received an injection of sodium chloride solution but no caffeine contained 0.49 per cent. The urine secreted after the injection of caffeine contained 0.86 per cent. of sodium chloride, while that secreted by control animals which merely received an intraperitoneal injection of sodium chloride solution contained only 0.46 per cent. Thus under the influence of caffeine a much larger quantity of sodium chloride has been eliminated from the body through the kidneys and we find that in such animals the blood and recovered peritoneal fluid contain less sodium chloride than in control animals.

In spite of the lower sodium chloride content of the blood in experiments in which caffeine was injected, we find after the absorption of sodium chloride solution almost no difference between the freezing points of the blood in normal animals that had received an injection of caffeine and those that had not received such an injection. In the former the average freezing point was —0.58°, in the latter it was —0.586°. The average osmotic pressure of the recovered peritoneal fluids was also the same in both cases; the freezing point was —0.59° when caffeine was injected and —0.593° in control experiments. Since the osmotic pressure of both peritoneal fluid and of blood of normal animals injected with caffeine was almost the same as in control animals, while the sodium chloride content of both blood and peritoneal fluid was lower than in control animals, more osmotically active substances other than sodium chloride must have entered the blood and peritoneal fluid under the influence of caffeine.

If our explanation of the main factor determining the variations in absorption of fluid from the peritoneal cavity be correct, we should not find an increase of the osmotic pressure of the blood after an injection of caffeine, inasmuch as caffeine did not cause an increase in absorption from the peritoneal cavity. We, therefore, injected into normal animals at half-hour intervals the usual doses of caffeine without, however, injecting into such rabbits sodium chloride solution. These rabbits were killed shortly after the fourth injection of caffeine, and the average freezing point of the blood serum was found to be —0.584°. In previous experi-

ments we have found the average freezing point of the blood of normal animals which had not been injected with either sodium chloride solution or caffeine to be —0.60°. The injection of caffeine caused, therefore, a slight diminution in the osmotic pressure of the blood serum and this decrease corresponds to a slightly lessened absorption of fluid from the peritoneal cavity.

As a result of the absorption of fluid from the peritoneal cavity, the blood of animals, which in addition to the caffeine had received an injection of sodium chloride solution, showed an average decrease of the refractive index of the blood serum of only .000033; in normal animals injected with sodium chloride solution without the addition of caffeine, the average decrease was .00133. In experiments with caffeine the amount of water retained in the blood vessels as a result of the absorption of the sodium chloride solution has, therefore, been considerably less than in control experiments. This decrease in the retention of fluid was due to the increased diuresis, but, in addition, we have to consider the possibility that an increased amount of fluid may pass from the blood vessels into the tissues, in much the same manner as we have noted the passing of fluid out of the vessels into the tissues after an injection of adrenalin.

Thus we find that the injection of caffeine slightly lowers the osmotic pressure of the blood of normal animals, and probably as a result of this lowering of the osmotic pressure of the blood the absorption of fluid from the peritoneal cavity is found to be slightly diminished. The absorption of sodium chloride from the peritoneal cavity is, on the other hand, increased, notwithstanding the relative decrease of the sodium chloride in the blood. Since in experiments in which caffeine was injected, not only the sodium chloride percentage, but also the bulk of the blood after the absorption of sodium chloride solution is less than in control experiments without the injection of caffeine, the absolute quantity of sodium chloride retained in the blood must be diminished to an even greater degree than would appear from a consideration of the sodium chloride percentage alone. Both lessened retention of fluid in the blood vessels and the lower sodium chloride percentage of the blood may be due to two factors, namely, the increased elimi-

nation of both salt and water by the kidneys, and a specific action of caffeine which causes these substances to pass out of the blood vessels into tissues other than the kidney.

In these experiments as well as in experiments with normal animals in which no caffeine had been injected, we find both the osmotic pressure and the sodium chloride percentage in the recovered peritoneal fluid higher than in the blood serum.

ABSORPTION IN ANIMALS INJECTED WITH DISTILLED WATER AND CAFFEINE.

In rabbits which, in addition to an intraperitoneal injection of 120 cubic centimeters of distilled water, received repeated injections of caffeine, we recovered an average of 35 cubic centimeters of fluid from the peritoneal cavity, whereas in animals which received an injection of distilled water, but not caffeine, 44 cubic centimeters of fluid were recovered. Caffeine has, therefore, slightly increased the absorption of distilled water, since under the influence of caffeine 85 cubic centimeters of fluid were absorbed, while in experiments without injection of caffeine 76 cubic centimeters of fluid were absorbed. In the animals injected with caffeine, an average of 38 cubic centimeters of urine was secreted, while 14 cubic centimeters were secreted by animals which received no caffeine. In animals injected with caffeine the diuresis was not so markedly increased when distilled water was injected as when 0.85 per cent. sodium chloride solution was used.

In these experiments, as in experiments with normal animals injected with sodium chloride solution, caffeine has diminished the sodium chloride percentage of the recovered peritoneal fluid, but did not, as in experiments in which sodium chloride solution was injected, diminish the sodium chloride percentage of the blood serum. The diminution of the amount of sodium chloride which passed into the peritoneal cavity in the animals which, in addition to distilled water, were injected with caffeine, is due to the same factors which caused the sodium chloride content of the peritoneal fluid and blood to be less in animals injected with caffeine in addition to sodium chloride solution, namely, to the increased elimination of sodium chloride through the kidneys and to some influence

of caffeine causing sodium chloride to pass into the tissues of the body.

It appears that the addition of caffeine has not markedly influenced the osmotic pressure of the peritoneal fluid or the blood serum in animals injected with distilled water. Since the peritoneal fluid in animals injected with caffeine and distilled water contained a lower sodium chloride percentage than the peritoneal fluid in animals that did not receive caffeine, and yet the osmotic pressure of the fluids was the same, relatively more osmotically active substances other than sodium chloride must have passed into and been retained in the peritoneal fluid of animals which received injections of caffeine. More osmotically active substances other than sodium chloride have passed from the tissue into the blood in animals injected with caffeine in addition to distilled water than in animals in which caffeine was not injected. This follows from the fact that notwithstanding the increased amount of urine containing a large amount of osmotically active substances, and the large amount of osmotically active substances passing into the peritoneal fluid, the osmotic pressure of the blood has not decreased.

In experiments in which caffeine was injected in addition to distilled water, the osmotic pressure of the peritoneal fluid was less than that of the blood, in a similar manner as in experiments in which caffeine was not used.

The amount of fluid retained in the blood of rabbits which, in addition to distilled water, received injections of caffeine, was slightly less than the amount retained in the blood of animals not injected with caffeine, as shown by the diminution of the refractive index of the blood serum, which was 0.00125 with caffeine as against 0.00136 without caffeine.

We see that after an intraperitoneal injection of distilled water caffeine did not cause as marked a diminution in the amount of water retained in the blood as after an injection of 0.85 per cent. sodium chloride solution. This may be partly due to the fact that, in experiments in which caffeine was injected in addition to distilled water, the absorption of water from the peritoneal cavity was increased and that the elimination of fluid through the kidneys was under such conditions somewhat less than in experiments in which

0.85 per cent. sodium chloride solution was used instead of distilled water. Both of these factors tend to increase the amount of water retained in the blood. There may be another factor present, namely, the distilled water may perhaps injure or interfere with the mechanism through which the caffeine decreases the retention of fluid in the vessels.

ABSORPTION IN NEPHRECTOMIZED ANIMALS INJECTED WITH CAFFEINE.

In these experiments we studied the influence of caffeine on absorption in animals, in which twenty-four hours previously the vessels and ureters of both kidneys had been ligated. After the intraperitoneal injection of 0.85 per cent. sodium chloride solution and the repeated injection of caffeine, in such animals we recovered an average of 28 cubic centimeters of fluid from the peritoneal cavity. Caffeine has distinctly increased the absorption of fluid in nephrectomized animals.

We have already shown that the osmotic pressure of the blood is an important, if not the most important, factor influencing the absorption of fluid from the peritoneal cavity. Could this increased absorption of fluid in nephrectomized animals be due to an influence of caffeine on the osmotic pressure of the blood? In order to determine the influence of caffeine on the osmotic pressure of the blood in nephrectomized animals, we injected caffeine into the peritoneal cavity of such animals every half hour, but omitted the injection of sodium chloride solution. In previous experiments we found the average freezing point of the blood serum of nephrectomized rabbits, which had not received caffeine, to be — 0.64°; in five nephrectomized animals which had received injections of caffeine, the average freezing point of the serum was — 0.70°.

We see, therefore, that the osmotic pressure of the blood is increased in nephrectomized animals which received caffeine; this increase represents the additive effect of nephrectomy and of the injection of caffeine. As we have seen, nephrectomy alone increases the osmotic pressure of the blood and the injection of caffeine in nephrectomized animals causes a marked additional rise of the osmotic pressure, while in normal animals caffeine does

not cause a rise in osmotic pressure. In normal animals the functionating of the kidneys seems to prevent the increase in the osmotic pressure of the blood under the influence of caffeine, while in nephrectomized animals the retention of substances which cannot be eliminated through the kidneys seems to cause an increase in the osmotic pressure of the blood.

Inasmuch as under the influence of caffeine the urine shows a relative decrease in the elimination of solids as compared with the elimination of fluid, this difference in the behavior of the osmotic pressure of the blood in normal and in nephrectomized animals under the influence of caffeine cannot alone be due to this increased elimination of fluid and solid substances from the blood into the urine in non-nephrectomized animals, since such an elimination in itself would rather tend to increase the osmotic pressure of the blood instead of diminishing it. If we assume that both fluids and solids pass directly from the blood into the urine, we have to assume that complicating factors come into play, in order to explain this difference in changes in the osmotic pressure of the blood in normal and in nephrectomized animals.

In agreement with our previous observations we again note a parallelism in the changes in the osmotic pressure of the blood and in the rate of absorption from the peritoneal cavity. In nephrectomized animals, caffeine increases the osmotic pressure of the blood and also increases the absorption of fluid, while in normal animals it has neither of these effects.

In nephrectomized animals the absorption of sodium chloride from the peritoneal cavity was increased by caffeine in the same way as in normal animals. We have seen that nephrectomy alone increases the absorption of sodium chloride from the peritoneal cavity and when caffeine is injected into such animals the absorption of sodium chloride is even more increased. In view of the lower sodium chloride percentage in the recovered peritoneal fluid and the greater amount of fluid absorbed in experiments with nephrectomized animals injected with caffeine, the absolute amount of sodium chloride absorbed from the peritoneal cavity is greater than in normal animals or in nephrectomized animals without the injection of caffeine. In normal animals which did not receive

caffeine, the absolute amount absorbed was 0.588 gram, in nephrectomized animals without caffeine it was 0.796 gram, and in nephrectomized animals with caffeine it was 0.857 gram.

Just as the sodium chloride percentage of the peritoneal fluid was lower in experiments with nephrectomized animals which received injections of caffeine, so the sodium chloride percentage of the blood serum was also lower than in nephrectomized animals which received no caffeine.

Since under the influence of caffeine the absorption of sodium chloride from the peritoneal cavity of nephrectomized animals is increased and the blood shows a lower sodium chloride percentage than in control experiments, more sodium chloride must have passed into the tissues than in experiments in which no caffeine was injected. We see, therefore, that when the kidneys are performing their normal function, caffeine causes an increased elimination of sodium chloride from the blood through the kidneys, and, if the kidneys do not functionate, caffeine still causes an increased amount of sodium chloride to pass out of the peritoneal fluid and blood. In the latter case it must be retained in the tissues generally or in a specific organ or tissue.

The average freezing point of the blood serum after the absorption of fluid from the peritoneal cavity was, in nephrectomized animals, — 0.623° when caffeine was injected, and it was the same in nephrectomized animals without the injection of caffeine. The average freezing point of the peritoneal fluid in nephrectomized animals injected with caffeine was — 0.623°, but without caffeine it was — 0.59°. Thus we find that, in spite of the differences in the sodium chloride content of the blood in the two series of experiments, the osmotic pressure is the same in nephrectomized animals with and without the injection of caffeine, and more osmotically active substances other than sodium chloride must, therefore, be present in the serum of nephrectomized animals injected with caffeine than in the serum of nephrectomized animals without caffeine. In nephrectomized animals injected with caffeine the osmotic pressure of the recovered peritoneal fluid is higher, while the sodium chloride percentage is lower than in nephrectomized animals without caffeine. Thus the amount of other osmot-

ically active substances which have passed into the peritoneal cavity in nephrectomized animals is markedly increased under the influence of caffeine. It appears that caffeine causes the osmotically active substances other than sodium chloride to be absolutely and relatively increased in both the blood and peritoneal fluid and the surplus of these substances must have been drawn from the tissues.

The injection of caffeine into nephrectomized animals caused an average lowering of the refractive index of the blood of 0.0010 after the absorption of sodium chloride solution. Under the same condition in nephrectomized animals not injected with caffeine, the lowering of the refractive index amounted to 0.0015. Here and in normal animals caffeine diminished the retention of fluid in the blood vessels. Since in these experiments no fluid left the body through the kidneys, and yet a larger quantity of fluid was absorbed from the peritoneal cavity than in non-nephrectomized animals without the injection of caffeine, it appears that caffeine causes fluid as well as sodium chloride to leave the vessels and to enter the tissues. Adrenalin, on the other hand, accelerates, as we have found, only the movement of fluid and not of sodium chloride from the blood into the tissues. The fact that under the influence of caffeine the fluid content of the blood after the absorption of a sodium chloride solution from the peritoneal cavity is less than in control animals without the injection of caffeine proves that in nephrectomized as well as in normal animals the elimination of sodium chloride caused by the injection of caffeine is even considerably greater than would be indicated by the figures of the sodium chloride content of the blood.

ABSORPTION UNDER THE INFLUENCE OF CAFFEINE IN OPERATED BUT NOT NEPHRECTOMIZED ANIMALS.

In this series of experiments rabbits were operated on twenty-four hours previous to the study of the absorption. In each animal two lumbar incisions were made and the peritoneum was exposed; during the operation the peritoneum was repeatedly touched by the operator's fingers, care being taken that the peritoneum and the kidneys should not be injured.

When in addition to the sodium chloride solution we injected

caffeine intraperitoneally into such animals, we recovered an average of 41 cubic centimeters of peritoneal fluid at the termination of the experiment; while in operated animals which received no injection of caffeine, an average of 57 cubic centimeters of fluid was recovered from the peritoneal cavity, as stated previously. An average of 48 cubic centimeters of urine was secreted by the animals which received caffeine, while 18 cubic centimeters was secreted by the animals which received no caffeine. Here as in nephrectomized animals the injection of caffeine increased the absorption of fluid from the peritoneal cavity, but, as we have not determined the influence of caffeine on the osmotic pressure of the blood in these animals, we cannot positively state that in this case also an additive increase of the osmotic pressure of the blood due to the injection of caffeine is the cause of the increased absorption from the peritoneal cavity. We are, however, very much inclined to believe this to be the case, inasmuch as in animals operated in the manner described above as well as in nephrectomized animals, the osmotic pressure of the blood is raised and inasmuch as we have found that the injection of caffeine in nephrectomized animals causes an additional rise in the osmotic pressure. In view of the fact that absorption is not so markedly increased after the administration of caffeine in animals in which a lumbar incision has been made as in nephrectomized animals after the injection of caffeine, it is very likely that in the former animals caffeine does not cause so marked a rise of the osmotic pressure of the blood as it does in nephrectomized animals.

In animals in which a lumbar incision was made and in which caffeine was injected, the sodium chloride percentage of the recovered peritoneal fluid was 0.46 per cent. and the absolute amount of sodium chloride absorbed was 0.84 gram on the average. The average sodium chloride percentage in the blood serum was 0.44 per cent. The sodium chloride percentages in both peritoneal fluid and blood serum were, therefore, very small, while the quantity of sodium chloride absorbed from the peritoneal cavity was large. The influence of caffeine in operated animals has been such as to cause more sodium chloride to leave both the peritoneal fluid and the blood serum and to pass into the tissues. This observation is

in perfect accordance with what we have noted in normal and in nephrectomized animals regarding the influence of caffeine on the movements of sodium chloride.

The average freezing point of the recovered peritoneal fluid in animals with a simple lumbar incision which received caffeine was —0.575°, and that of the blood serum was —0.595°. This relation of the osmotic pressure of the peritoneal fluid to that of the blood serum was the same as in all other cases in which the osmotic pressure of the blood was raised above the normal level under the influence of experimental procedure. If we compare the results obtained in the case of operated animals which received caffeine with the results obtained in normal animals injected with caffeine, we note that after the absorption of fluid the osmotic pressure of the blood in operated animals injected with caffeine was somewhat higher than that of the blood of normal animals injected with caffeine; this is in conformity with the fact which we have pointed out in a previous paper that lumbar incision increases the osmotic pressure of the blood. While, therefore, the osmotic pressure of the blood in operated animals injected with caffeine was higher than that of the blood of normal animals injected with caffeine, the sodium chloride content was lower in operated animals; a relatively greater quantity of other osmotically active substances must, therefore, have passed from the tissues into the blood of these animals. Both the osmotic pressure and the sodium chloride content of the recovered peritoneal fluid of operated animals injected with caffeine was somewhat lower than that of normal animals which were injected with sodium chloride solution alone; the difference between the sodium chloride content, was, however, much greater than the difference between the osmotic pressure, so that in the peritoneal fluid of operated animals injected with caffeine, as well as in the blood serum of these animals, a large quantity of osmotically active substances other than sodium chloride was present. This is in agreement with the results noted in the other series of experiments in which caffeine was injected.

The decrease in the refractive index of the blood serum of operated animals injected with caffeine was 0.000033, while in operated animals which received no caffeine the average decrease

was 0.0015. Just as in normal animals, so in operated animals the amount of fluid retained in the blood vessels is diminished under the influence of caffeine. This is due to the increased diuresis caused by the administration of caffeine, but it may also be due in part to an increased output of fluid from the blood into the tissue in a similar way as we have observed an exchange of fluid in nephrectomized animals which received caffeine. We find, therefore, that in operated animals caffeine increases the absorption fluid from the peritoneal cavity and also the secretion of urine. It likewise increases the withdrawal of sodium chloride from both the peritoneal cavity and blood and causes a movement of other osmotically active substances into the blood and peritoneal cavity.

INFLUENCE OF CAFFEINE ON ABSORPTION IN ANIMALS INJECTED WITH URANIUM NITRATE.

In animals which received an injection of five milligrams of uranium nitrate three days before the absorption experiment, an average of 92 cubic centimeters of fluid were recovered from the peritoneal cavity after the intraperitoneal injection of sodium chloride solution and of caffeine. In experiments with animals injected with uranium nitrate which did not receive caffeine, 77 cubic centimeters of fluid were recovered, as we stated in a previous publication. The amount of urine is not increased by the injection of caffeine into such animals. Here, as in normal animals, caffeine has not only not increased but has somewhat decreased the rate of absorption from the peritoneal cavity.

In animals injected with uranium nitrate the freezing point of the blood serum after the absorption of fluid from the peritoneal cavity is slightly higher in experiments in which caffeine had been injected than in experiments in which no caffeine was injected. The average freezing point in the experiments with caffeine was 0.645°, and in experiments without caffeine, 0.63°.

The injection of caffeine in addition to sodium chloride solution into animals which had been injected with uranium nitrate causes an average reduction of the refractive index of the blood serum of 0.0012. In animals injected with uranium nitrate which did not receive caffeine the average reduction was 0.0022. In animals

which were injected with uranium nitrate three days previously caffeine causes, therefore, less fluid to be retained in the blood than in animals treated with uranium nitrate which received no caffeine. This may be partly due to the smaller amount of fluid which was absorbed after the administration of uranium nitrate, but caffeine again causes in all probability, even in uranium nitrate animals, fluid to pass out of the vessels and into the tissues.

We note, therefore, that in animals which had been injected with uranium nitrate three days previously caffeine has no accelerating effect on absorption in spite of the high osmotic pressure of the blood. The injection of uranium nitrate has brought into play some factor counteracting the influence of the high osmotic pressure of the blood on absorption from the peritoneal cavity; this we have already pointed out in our previous article on the influence of uranium nitrate. This factor may possibly consist of a change in the permeability of the endothelial cells of the blood vessels and peritoneum brought about under the influence of uranium nitrate.

DISCUSSION OF RESULTS.

Adrenalin and caffeine differ in some respects in their influence on absorption from the peritoneal cavity. Thus in normal animals adrenalin increases absorption, caffeine decreases it; in nephrectomized animals caffeine markedly increases absorption, but adrenalin increases it only slightly, if at all; in normal animals which were injected intraperitoneally with distilled water, adrenalin markedly increases absorption, caffeine increases very slightly the rate of absorption. In animals which have been injected with uranium nitrate three days previously adrenalin increases, while caffeine decreases the absorption of fluid.

In all series of experiments, the injection of caffeine causes less fluid to be retained in the blood vessels than in the corresponding control experiments. In some of these experiments the diminished retention of the fluid may be due to the increased diuresis, but certainly in nephrectomized animals and animals injected with uranium nitrate three days previously, there must exist some factor, which causes the fluid to pass from the blood vessels into the tissues. A similar movement of fluid from the blood into the tissues was noted in experiments in which adrenalin was injected.

In all series of experiments in which caffeine was injected the sodium chloride percentages of the recovered peritoneal fluid and of the blood serum were lower than in animals which had not rceived caffeine. Under the influence of caffeine the movement of sodium chloride follows, therefore, in the same direction which we found water to follow after administration of caffeine. Both salt and water pass from the blood into the tissues and the sodium chloride passes also from the peritoneal cavity into the tissues, either directly or indirectly.

The increased diuresis caused by caffeine is generally explained as being due primarily to an increased elimination of sodium chloride through the kidneys. *Our experiments show that, under the influence of caffeine, sodium chloride passes out of the blood, even after the removal of the kidneys. When the kidneys are functionating, this salt is forced out of the body through the kidneys, but when the functions of the kidneys are entirely suppressed or when renal insufficiency exists, an increased quantity of sodium chloride passes out of the blood and is taken into the tissues under the influence of caffeine.* The action of caffeine in increasing the sodium chloride elimination seems, therefore, not necessarily to be due to an action of caffeine upon the kidneys. *The primary condition underlying the caffeine diuresis seems to be the elimination of sodium chloride from the blood, and the kidney appears to play only a secondary rôle in this process.*

In contra-distinction to the relative diminution of sodium chloride in the blood and the peritoneal fluid under the influence of caffeine, other osmotically active substances in both blood and peritoneal fluid of animals injected with caffeine must be increased, since, as stated above, the osmotic pressure of these fluids is identical with that found in animals which did not receive caffeine. Large quantities of these other substances must have passed from the tissues into the blood. It may be that caffeine increases the metabolic activity and that as a result of this increase more osmotically active substances are formed in the tissues. This would not, however, explain the movement of sodium chloride into the tissues; there must be some specific condition which is unknown to us at the present time causing the passing of the latter substance from

the body fluids into the tissues. After the injection of adrenalin, on the other hand, a change in the movement of sodium chloride and of other osmotically active substances is usually absent, or if changes in the distribution of sodium chloride and other osmotically active substances are noted, they are not sufficiently regular and they do not permit us, therefore, to attribute to adrenalin a definite influence upon the distribution of these substances.

Several previous investigations have been reported concerning the influence of diuretic substances belonging to the caffeine groups on absorption. In all of these experiments the investigators merely tested this influence in either nephrectomized animals or in animals poisoned with uranium nitrate, and the conclusions at which they arrived are, therefore, at variance with the conclusions to be drawn from the results of our experiments.

Weber (3) studied the influence of theophyllin on the absorption of sodium chloride solutions from the subcutaneous tissue in nephrectomized animals. In control experiments he found that after an intravenous injection of theophyllin in nephrectomized animals in which no sodium chloride solution had been injected subcutaneously, the water as well as the chlorides and the ash in general were increased in the blood. When he injected into nephrectomized animals isotonic salt solutions subcutaneously and theophyllin intravenously, the water, ash and chloride contents of the blood were likewise increased; when he injected a hypertonic solution of sodium chloride subcutaneously the intravenous injection of theophyllin also increased the ash and chloride content of the blood, while, on the other hand, the blood became more concentrated than in control experiments in which theophyllin was not injected.

Weber found that under the influence of caffeine the sodium chloride content of the blood is increased, whereas we found the quantity of this salt in the blood to be diminished. This investigator found also that the fluid in the blood was increased when caffeine was injected, except in cases in which hypertonic solutions were used, while we found throughout our experiments that caffeine decreased the fluid content of the blood. On the other hand, our results coincide with Weber's, inasmuch as he found that the ash

of the blood was increased by the injection of caffeine and we have found that caffeine increases the amount of osmotically active substances other than sodium chloride in the blood.

One objection perhaps can be raised against Weber's experiments, inasmuch as he withdrew comparatively large quantities of blood from his animals in the beginning of the experiment. Furthermore, his experiments are not directly comparable with ours, inasmuch as he studied the absorption from the subcutaneous tissues, while we studied the absorption from the peritoneal cavity.

Georgopulos (4) injected ascitic fluid which he obtained from animals poisoned with uranium nitrate into the peritoneal cavity of nephrectomized rabbits and found that under the influence of caffeine the absorption of fluid was hastened.

From these experiments both of these observers drew the conclusion that diuretic substances of the caffeine group cause an increase in the rapidity of absorption, and in order to explain this phenomenon they introduced a so-called vital factor, assuming that these diuretic substances exerted a direct stimulating effect on the endothelial cells of the body and thus caused a more rapid movement of substances from the peritoneal cavity or tissues into the blood vessels. As we stated above, we explain the influence of caffeine on absorption in an entirely different manner and we believe that the previous investigators failed to note the influence of caffeine on the osmotic pressure of the blood in its relation to absorption because they did not carry out experiments concerning the influence of these substances on absorption in normal animals. This they probably omitted to do because they tacitly assumed that, inasmuch as in normal animals caffeine increases so very markedly the diuresis, it was *a priori* certain that the absorption would also be markedly increased under such conditions. Our experiments show, on the contrary, that the opposite effect is associated with the increased diuresis caused by caffeine in normal animals.

Both Georgopulos (4) and Richter (5) have found that repeated injections of caffeine or diuretics of a similar nature into animals poisoned with uranium nitrate diminished the degree of edema which was found at the end of the experiment. These observers believe, therefore, that these substances stimulate cells whose

function it was to absorb fluid from the tissues or from the peritoneal cavity. Their experiments were very complicated, inasmuch as they extended over several days and over various experimental periods, in which the conditions of the animals must have varied considerably, and if increased absorption actually took place under such conditions, it probably can be ascribed to an increase in the osmotic pressure of the blood which existed at some period of the experiment. Another possible explanation of their results, however, is the following: in their experiments the influence of caffeine in diminishing the edema was not a direct effect upon absorption, but their results were due to the fact that, in the earlier periods of their experiments, caffeine caused an increased elimination of the fluid which had been introduced into the stomachs of the animals. Inasmuch as the quantity of edematous fluid present in animals injected with uranium nitrate depends upon the amount of fluid available in the body, it necessarily follows that if considerable quantities are removed from the body through an increase in diuresis, less fluid becomes available for the formation of edema. In this case the influence of diuretics on the formation of edema is, therefore, an indirect one and is not due to a stimulating action which, according to the above named authors, diuretics of the caffeine group exert on absorption. According to our experiments, caffeine increases absorption only if it raises the osmotic pressure of the blood, but it may decrease edema by diminishing the fluid available for the formation of edema.

While we are thus able to explain the influence of caffeine and similar diuretics on absorption of water, the changes in the distribution of sodium chloride and of other osmotically active substances under the influence of these drugs, at the present time we can merely describe these changes without being able to state their cause. Hypothetically we might, however, suggest that certain metabolic processes which take place in the tissues under the influence of caffeine are mainly responsible for the changes in the distribution of sodium chloride and other osmotically active substances.

Throughout all our experiments in which we have studied the effect on absorption from the peritoneal cavity of various experimental conditions, such as the injection of adrenalin or caffeine,

nephrectomy, or other operative interferences, and various combinations of these conditions, we found in all cases, in which the necessary determinations have been made, that the changes in the osmotic pressure of the blood induced by these experimental conditions were sufficient to explain the changes in the rate of absorption. Thus adrenalin increased the osmotic pressure of the blood and increased the absorption of fluid, while caffeine decreased the osmotic pressure of the blood in normal animals and diminished the rate of absorption. In nephrectomized animals both the osmotic pressure of the blood and the rate of absorption from the peritoneal cavity were increased, and in such animals caffeine caused an additive increase in the osmotic pressure of the blood and caused correspondingly a still greater amount of fluid to be absorbed from the peritoneal cavity. Not only nephrectomy but other operations also increased the osmotic pressure of the blood and thus increased the rate of absorption. In animals which were etherized we found no increase in the osmotic pressure of the blood and consequently the rate of absorption was unchanged.

That increased diuresis bears no causal relation to the increased absorption of fluid becomes evident if we consider the effect of caffeine on absorption in normal animals. In spite of the markedly increased amount of urine secreted, the amount of fluid absorbed from the peritoneal cavity is diminished in experiments with normal animals injected with caffeine. On the other hand, in normal animals which received an intravenous injection of adrenalin, the amount of urine secreted is the same as in control animals which received no adrenalin, but, nevertheless, the absorption of fluid is increased.

At present we do not wish to state that the osmotic pressure of the blood is the only factor determining the rate of absorption of fluid from the peritoneal cavity. We have pointed out certain conditions in which complicating factors arise, as in animals injected with uranium nitrate and in animals affected with peritonitis. Leaving out of consideration these and other cases of a complicated character, we may state that in our experiments changes in the osmotic pressure of the blood are sufficient to explain the principal variations in the rate of absorption which we have noticed.

We add here two tables, in the first of which we show the relations existing between the osmotic pressure of the blood, the diuresis and the absorption of fluid from the peritoneal cavity; in the second we give the averages of the results obtained in all our experiments concerning absorption from the peritoneal cavity.

TABLE I.

Relations between the Osmotic Pressure of the Blood, Diuresis and Absorption of Fluid from the Peritoneal Cavity. (Compared with the Results Obtained in Normal Animals Injected with 0.85 per cent. Sodium Chloride Solution.)

Experimental condition.	Osmotic pressure of blood.	Diuresis.	Absorption of fluid.
Injection of adrenalin	+	+	+
Injection of caffeine	—	+	—
Nephrectomy	+	O	+
Various operations other than nephrectomy	+	slight + or =	+
Etherization	=	=	=
Nephrectomy and caffeine	++	O	++
Injection of uranium nitrate one day previously	+	+	=
Injection of uranium nitrate three days previously	+	—	=
Peritonitis	+	= or —	—

(+ increased; — decreased; = unchanged; O absent; ++ markedly increased.)

Certain phenomena which we observed in our experiments we cannot explain at the present time. For instance, in animals injected with uranium nitrate the osmotic pressure of the blood is increased, but the rate of absorption of fluid from the peritoneal cavity is not increased. The cause of the changes in the distribution of sodium chloride or other osmotically active substances in nephrectomized animals, in animals poisoned with uranium nitrate, or in animals injected with caffeine, is also at present obscure. Before we turn for an explanation of these phenomena to the assumption of the so-called vital activity of the endothelial cells of the peritoneum or of the blood vessels, we prefer to wait until the analysis of the changes occurring under the various experimental conditions which we produced in our investigations shall have been carried much farther, and, for the present, to suspend judgment.

TABLE II.

Average Results of Experiments on Absorption from the Peritoneal Cavity.

	Blood before experiment.		Recovered fluid.						Serum.			Urine.	
	Sodium chloride per cent.	Δ	Amount in c.c.	Sodium chloride per cent.	Sodium chloride absorbed in grms.	Sodium chloride absorbed pro c.c.	Δ	Refractive index.	Sodium chloride per cent.	Δ	Dilution.	Amount in c.c.	Sodium chloride per cent.
Normal ; 0.85 per cent. sodium chloride	.455	—0.60°	77	.53	.588	.014	—0.593°	1.3353	.49	—0.586°	—.00133	11	.46
Normal ; 0.85 per cent. sodium chloride ; adrenalin intraperitoneally	—	—0.62	41	.52	.811	.010	—0.593	1.3354	.47	—0.586	—.00065	41	.53
Normal ; 0.85 per cent. sodium chloride ; adrenalin intravenously	—	—	62	.58	.72	.011	—0.593	—	.52	—0.593	—	11	.51
Normal ; distilled water	—	—	44	.40	—	—	—0.57	1.3361	.41	—0.583	—.00136	14	.32
Normal ; distilled water ; adrenalin intraperitoneally	—	—	21	.36	—	—	—0.553	1.3359	.41	—0.58	—.0012	49	.40
Normal ; 1.5 per cent. sodium chloride	—	—	116	.59	1.1565	.2891	—0.634	1.3352	.55	—0.648	—.00026	8	.92
Normal ; 1.5 per cent. sodium chloride ; adrenalin intraperitoneally	—	—	81	.62	1.2852	.0357	—0.654	1.3353	.51	—0.656	—.00036	30	.92
Nephrectomy	.466	—0.64	47	.505	.796	.010	—0.59	1.3361	.487	—0.62	—.0015	0	—
Nephrectomy + adrenalin	—	—	43	.49	.861	.0108	—	1.3364	.49	—	—.00105	0	—
1 day uranium nitrate	.495	—0.615	74	.585	.606	.012	—0.59	—	.54	—0.60	—.0015	34	.41
1 day uranium nitrate + adrenalin	—	—	46	.60	.794	.0095	—	—	.54	—	—.0005	64	.55
3 day uranium nitrate	.466	—0.675	77	.595	.587	.013	—0.60	1.3364	.505	—0.63	—.0022	4	—
3 day uranium nitrate + adrenalin	—	—	64	.57	.624	.011	—	1.3358	.50	—	—.0022	6	—
Etherization	—	—0.60	70	—	—	—	—	—	—	—0.59	—	20	—
Lugol's solution	—	—	71	—	—	—	—	—	—	—	—	13	—
Lumbar incision	—	—0.624	57	—	—	—	—	—	—	—	—.0015	18	—
Abdominal incision	—	—0.663	54	—	—	—	—	—	—	—0.64	—	—	—
Thoracic incision	—	—0.665	53	—	—	—	—0.609	—	—	—0.635	—.0009?	18	—
Peritonitis	—	—	133	—	—	—	—0.66	—	—	—0.71	—	—	—
Normal ; caffeine	—	—0.584	82	.47	.669	.015	—0.59	1.3358	.46	—0.583	—.000033	67	.86
Distilled water and caffeine	—	—	35	.32	0	—	—0.575	—	.41	—0.583	—.00125	38	—
Nephrectomy and caffeine	—	—0.70	28	.47	.857	.010	—0.623	1.3354	.47	—0.623	—.0010	0	—
Lumbar incision and caffeine	—	—	41	.46	.84	.010	—0.575	—	.44	—0.595	—.000033	48	—
3 day uranium nitrate and caffeine	—	—	92	—	—	—	—	1.3360	—	—0.645	—.0012	—	—

CONCLUSIONS.[2]

I. In normal animals the injection of caffeine slightly diminishes the absorption of fluid from the peritoneal cavity, in spite of the fact that the amount of fluid and sodium chloride eliminated through the kidneys is markedly increased. The lessened absorption of fluid is due to a slight lowering of the osmotic pressure of the blood.

II. In nephrectomized animals caffeine increases the absorption of fluid from the peritoneal cavity; the increase in absorption is greater in nephrectomized animals which received caffeine than in nephrectomized animals which did not receive this substance, and it is due to additive increase in the osmotic pressure of the blood. In a similar manner, caffeine increases the absorption of fluid from the peritoneal cavity in animals in which, instead of nephrectomy, other operations, not directly affecting the kidneys, had been performed. In this case also the increase in absorption is presumably preceded by and due to an increase in the osmotic pressure of the blood.

III. In animals injected with uranium nitrate three days previously, caffeine diminishes the absorption of fluid from the peritoneal cavity, notwithstanding the high osmotic pressure of the blood which we observe in such animals. This agrees with the results of our previous experiments in which we found that in animals injected with uranium nitrate the absorption of fluid is not increased in spite of the rise of the osmotic pressure of the blood.

IV. At the time of the conclusion of the absorption experiments, the amount of fluid retained in the vessels was found to be diminished in each series in which caffeine was used. Only in certain cases can this be due to the increased amount of fluid leaving the blood vessels through the kidneys; in other cases it can only be due to a movement of water from the blood vessels into the tissues caused by the injection of caffeine.

V. In normal animals, in nephrectomized animals and in animals in which an operation not directly affecting the kidneys had been performed, caffeine causes an absolute and relative increase in the

[2] In the series of experiments reported in this communication, 101 rabbits were used in a corresponding number of individual experiments. The protocols of the individual experiments have been omitted.

elimination of sodium chloride from the peritoneal fluid, as a result of which the remaining peritoneal fluid shows a lessened content of sodium chloride. Caffeine causes also a decrease in the sodium chloride content of the blood. We see, therefore, that under the influence of caffeine a greater amount of sodium chloride is eliminated from the body fluids into the tissues or through the kidneys. The factors which cause the sodium chloride to leave the body fluids are probably primarily responsible for the diuresis which takes place after administration of caffeine. In the case of caffeine and other similar substances the diuresis is, therefore, in all probability not due primarily to a specific action of the kidney, but to conditions which affect the distribution of sodium chloride in the body.

VI. The distribution coefficient of other osmotically active substances differs from that of sodium chloride. These other substances have a tendency to move into the body fluids in increased quantities under the influence of caffeine.

VII. Summarizing all experiments in which we studied the absorption from the peritoneal cavity, we may state that changes in the osmotic pressure of the blood represent the principal factor in explaining the variations in the rate of absorption of fluid from the peritoneal cavity.

VIII. There exists no direct relation between an increase in the rate of absorption of fluid from the peritoneal cavity and an increase in the amount of urine secreted. If it should be found that even at a period following the injection of caffeine, later than that at which we have studied the absorption, a rise of the osmotic pressure of the blood does not appear, then we may state that the diminution in the amount of edema in the body cavities resulting from the administration of caffeine is entirely due to an inhibition of the production of edema and not to an increased absorption of fluid from the serous body cavities.

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OBSERVATIONS ON THE ORIGIN, DISTRIBUTION AND SIGNIFICANCE OF FUCHSIN BODIES, WITH SPECIAL STAINING TECHNIQUE.[1]

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Since the first description of fuchsin bodies by Fox (4) in 1858, an extensive literature upon the subject has been gradually accumulating and still, after fifty years, but little real progress has been made in the solution of their true pathological significance. Such widely divergent views are maintained by the leading workers that it is practically impossible for one to gain any clear conception of the subject from a review of the literature.

Though numerous opinions have, from time to time, been advanced to explain the origin and nature of fuchsin bodies, the general trend has been to narrow the question to two more general hypotheses.

I. Origin from cell granules.
   1. Plasma cells.
      a. Degeneration of "granuloplasm," (Unna (17)).
      b. Plasma cell granules, (Schridde (12), Fabian (2)).
   2. Origin from other granular cells, (leucocytes or *Mastzellen,* Lubarsch (7), Munter (8), and others).

II. Origin from red blood corpuscles, (Saltikow (11), Touton (16), Thorel (15), and others).

Detailed discussions of these hypotheses, with bibliography, may be found in several of the recent articles (3, 8). The object of this investigation has been to attempt to clear up some of the more fundamental questions which seem to have been a source of confusion. I refer, in particular, to the conception of what shall be considered a fuchsin body, the distribution and the relations of such bodies to other elements, and finally to the origin and significance of fuchsin bodies.

[1] Received for publication March 2, 1910.

The material for the present investigation comprises too extensive a list to publish in detail. It includes over two hundred distinct specimens representing nearly all the organs of the body and a great variety of pathological conditions as well as some normal tissues. The following list will give a general idea of the material used:

1. Tumors—benign and malignant	100
2. Inflammatory conditions—acute, chronic, abscesses, and organizing exudates	54
3. Vascular lesions—haemorrhages, thrombi, and infarcts.	16
4. Lesions of Spleen (7 types)	18
5. Lesions of lymph glands (6 types)	21
6. Lesions of liver (9 types)	9
7. Lesions of kidney (4 types)	6
8. Corpora lutea (ovary)	6
9. Goitres	3
10. Lesions of brain (3 types)	3
Total	236

This material was selected partly at random, and partly from lesions in which I felt sure of finding fuchsin bodies. The tissues were fixed by various methods, including Zenker's fluid, Orth's fluid, alcohol (95 per cent.), and formalin (10 per cent.). Paraffin and celloidin sections have been studied after staining with hematoxylin and eosin, van Gieson's stain, Weigert's fibrin stain, and Weigert's myelin sheath stain (Pal modification), as well as by a staining method which I have developed for this particular purpose. Of the staining methods mentioned above, the Weigert stains give by far the best results, though they are not adopted to routine work, while the other stains lack sharpness of definition and contrast. It is worthy of note that none of the stains recommended for fuchsin bodies is absolutely constant in reaction, unless it be hematoxylin and eosin.

The technique which I have employed for routine work meets some of these difficulties. It is relatively easy of application, gives exceedingly sharp contrast stain and, in my hands, is more constant in reaction than other methods.

Tissues are fixed in 10 per cent. formalin or 95 per cent. alcohol, formalin being preferable. Paraffin or celloidin sections are stained strongly in Delafield's hematoxylin. Stain five minutes in a 2 per cent. aqueous solution of acid fuchsin. Transfer to Lugol's solution for five to ten seconds. Wash quickly in water or 95 per cent. alcohol to remove iodine. Stain five to twenty seconds in a saturated aqueous solution of orange "G." Dehydrate with alcohol. Clear with any clearing reagent.

By this method, fuchsin bodies, red corpuscles (formalin fixed), and eosinophile granules stain a brilliant cherry red; nuclei, blue; cell protoplasm, buff to orange. No other type of hyaline ever takes the red stain and no other tissue element regularly takes this stain. Keratinized epitheleum, Mastzellen granules, and zymogen granules, occasionally stain red; fibrin stains red after alcohol fixation. All blood stained tissues stain red.

In using this stain, the treatment with Lugol's solution may be omitted and

the section washed until it has a faint pink tint, then the usual method is followed. The red stain is not usually so brilliant after this procedure. Again better results are sometimes obtained when the iodine is completely removed with alcohol before staining with orange "G," while a saturated alcoholic solution of orange "G" gives improved results with some tissues. The process of differentiation constitutes the one, though slight, difficulty of the method.

This technique can be recommended for demonstrating blood elements in tissues after formalin fixation and it is an excellent fibrin stain after alcohol fixation. It is not applicable for any purpose after Zenker's or Orth's fluid.

By reference to the literature, one may see that the bodies described as fuchsin bodies, by various authors, and most commonly, though probably improperly, designated as "Russell's fuchsin bodies," do not agree in all particulars, and this has undoubtedly given rise to much confusion. In considering the various types of fuchsin bodies (if there be such), it might be well to state that there is one form in the description of which all authorities agree. These bodies are round or oval on section, varying in size from that of an eosinophile granule to two to three times the diameter of a red blood corpuscle. They are highly refractile, and sharply contoured, occurring singly or, more commonly, in clusters of variable numbers and variable sizes, both intra and extracellularly. They show a strong affinity for acid stains. Such, in brief, may be considered the typical fuchsin body.

In a recent paper Fick (3) considers only such bodies as are round or oval, sharply contoured, and staining yellow with van Gieson's stain as true fuchsin bodies. Unna (18), on the other hand, has described a variety of forms, including columnar, prismatic, cuboidal and other shapes. Likewise there has arisen some difference of opinion in regard to staining reaction.[2]

Some claim that the typical fuchsin body stains yellow with van Gieson's stain; others, that it stains orange; and still others, that it stains red, excluding all bodies not staining the typical color. The same is true with other staining methods. This seeming discrepancy is due, in part, to composition of stains used by different workers and, in part, to the nature of the body in question, as perhaps modified by fixation. Most authorities agree that van Gieson's method stains fuchsin bodies yellow. What then of those bodies typical in morphology and not in staining reaction? Under

[2] For discussion of this question see Fick, *loc. cit.*

the term "fuchsin body," is one to include only those typical in both morphology and staining reaction or is the type subject to variation in one or both respects? This is naturally the first question which presents itself. It can be answered only after studying fuchsin bodies from a great variety of conditions by various methods of fixation and carefully checking one method of staining against the others. This gives the first reason for the extensive list of material I have used for this work, and the second is that I have been unable to find any article which has considered the question from so broad a field.

Considering first the question of morphology, I have found that by far the most common type of fuchsin body, after all methods of fixation and staining, is the oval or rounded, sharply contoured form. In many cases, this is the only form present. In other specimens, however, I have observed hyaline bodies of irregular shapes, though staining typically by all methods, and associated with typical fuchsin bodies. Further, the groupings and distribution were identical with those of typical bodies and, in some instances, both types could be observed in a single group. In still other cases, I have observed definite hexagonal plates and prisms, similarly associated with the typical forms and in no wise to be distinguished from them except in point of morphology. In one case (chorio-epithelioma) these hexagonal plates and prisms were the predominant forms. The prisms varied from very minute crystals to bodies measuring forty micra in length and from twelve to fifteen micra in diameter. The hexagons were apparently cross sections of the prisms.

In a majority of the cases in which the morphologically atypical fuchsin bodies were observed, bodies could be found which might be grouped in a series of gradually increasing irregularity from those showing only slight degrees of irregularity to those with markedly irregular morphology. In such a series, so gradual are the transitions that one must either consider all forms as fuchsin bodies, or confine his term to those which show no irregularity whatever. The latter position seems, to me, manifestly unjustifiable and I feel confident in stating that it is possible for fuchsin bodies to exhibit an exceedingly wide range of morphological variations.

As regards staining reactions, extended experience with the stains usually employed for demonstrating fuchsin bodies has convinced me that we can, with certainty, differentiate the fuchsin body type of hyaline from all other hyaline formations. I consider this particularly true of the triple staining method which I have employed. Yet, in any section showing fuchsin bodies in abundance, it is rare to find them absolutely uniform in type and degree of staining reaction. Most commonly, certain bodies manifest a variation in intensity of staining, taking a fainter or heavier stain than the average body of approximately the same size. This is observed even in bodies of the same cluster or in the same cell. Again, there are bodies, morphologically of the fuchsin body type, which take in varying degrees a different type of stain. For example, with van Gieson's method, some bodies will stain constantly orange or pink, or, by my triple staining method, instead of the fuchsin stain, they will take the orange "G." At first, I have considered these variations as defects of the staining methods, but they have been so constant, even after repeatedly restaining the section and controlling one method by another, that I feel sure there are bodies which will constantly manifest these atypical reactions. The same types of variation are noted among the red blood corpuscles in the same specimen.

Another interesting group of bodies, which I have not seen described elsewhere, are those with "basophilic" or "polychromatophilic" reactions. It is manifestly a misnomer to speak of these as fuchsin bodies. Still, they have some properties in common. They are morphologically identical with fuchsin bodies, very refractile, show identical distribution and groupings, and even occur in plasma cells along with perfectly typical fuchsin bodies. With hematoxylin and eosin, van Gieson's stain, and with "hematoxylin —acid fuchsin—orange G," they invariably stain blue or purple. I have observed these in only a few specimens and am undecided as to their correct interpretation. They are, however, not the nuclear fragments so often seen in necrotic areas and can be easily distinguished from such. In one specimen of hypernephroma, these "polychromatophile" hyaline droplets were very numerous and constituted the predominant type.

That the term "fuchsin body" is a misnomer for all these bodies with atypical staining reactions is evident, yet, it seems highly probable that many of them are closely related to typical fuchsin bodies. They are possibly of like origin and have suffered some change, either physical, or chemical, which is responsible for their atypical staining reactions.

Before leaving this phase of the question I wish to emphasize the occurrence of cells, with granules of a "fuchsinophilic" nature, in association with fuchsin bodies.[3]

The occurrence of such cells has been frequently pointed out. It is not constant, but frequent, and the point which has impressed me most forcibly is that in many conditions where one would expect to find fuchsin bodies in abundance, we may find only a few, and those usually small, while the section may be crowded with cells with granules of a fuchsinophilic character. The cells may be, in some cases, largely polymorphonuclear, though they are as commonly mononuclear cells of various types, principally of the plasma cell type. The cell granules vary in size from those as small as neutrophile granules to granules larger than those of the eosinophile of the blood, gradually shading into definite fuchsin bodies. The gradation in size and the identity in staining reaction is such that it is difficult to draw a line of separation between cells with fuchsinophile granules and fuchsin bodies. This difficulty is increased when both granules and fuchsin bodies exist in cells of the same class.

Therefore, as regards both morphology and staining reactions of fuchsin bodies, we undoubtedly have variations from the typical, and these are of such a nature as to render it very difficult to establish sharp lines of demarcation. There is the typical group, easily identified; there are bodies, morphologically atypical, but recognizable by staining reaction and association with typical bodies; there is a third group, atypical in staining reaction but typical in mor-

[3] These cells are usually referred to as eosinophile cells. I use the term "fuchsinophilic" to avoid confusion with the true eosinophile cell, as I do not consider it certain whether or not these cells are identical with true eosinophiles. I am inclined to doubt that many of them are identical with these cells. Some are probably Mastzellen, the granules of which will occasionally stain by my method.

phology and grouping. The significance of these two possible atypical groups will be considered later.

Leaving this phase of the question for the present, I will consider briefly the distributions and relations of fuchsin bodies. The literature contains numerous monographs on the occurrence of fuchsin bodies in tumors, gastric conditions, rhinoscleroma, as well as a number of other conditions, where this question is considered in detail, and I shall discuss only such points as appear to have some direct bearing on our interpretation of the fuchsin body.

The first point to be noted is that there is no single pathological condition, or no class of conditions, in which fuchsin bodies occur exclusively. Reference to the appended list of material will serve to show how widespread this occurrence is. The distribution of fuchsin bodies in tissues has been the subject of much discussion. Early observers described them in cells of granulation tissue. To a certain extent, this observation has received abundant confirmation, in so far that it is in granulation tissue and "granulation tissue areas" of tumors and other pathological conditions, if not actually in the connective tissue cells that these bodies occur most abundantly. Particularly is this true of areas rich in plasma cells. Within such areas, fuchsin bodies occur singly or in clusters; some are extracellular, while many of the plasma cells are filled with droplets of various sizes. The relation to plasma cells is so marked that, while all recognize it, some have advanced the idea that fuchsin bodies never occur except in relation to plasma cells.

However, other competent observers have described them in association with numerous cell types. For example, Klien (5) has described fuchsin bodies in connective tissue cells, Lubarsch (7) in association with *Mastzellen* and polymorphonuclear leucocytes, Sternberg (14) and Munter (8) in polymorphonuclear leucocytes, Konstantinowitsch (6) in endothelial cells, Askanazy (1) as hyaline metamorphosis of lymphocytes, and still others might be mentioned. Saltikow (11), Touton (16), Thorel (15), and others have described fuchsin bodies within the lumina of blood vessels.

I have been able to confirm most of these observation and, while the occurrence of fuchsin bodies within cells other than plasma cells is comparatively rare, there is no question but that perfectly

typical fuchsin bodies do occur in other types of cells. In the series of specimens I have studied, I have found typical fuchsin bodies in the following types of cells: plasma cells, most numerously; connective tissue cells, large mononuclear cells; more rarely, in cells of epithelial tumors, of adrenal tumors, of round cell sarcomata, and of endotheliomata; in liver cells in four cases; in stratified squamous epithelium in two specimens; in epithelium of kidney tubules in one case; in polymorphonuclear leucocytes in one case. Further, this series includes several sections in which no plasma cell areas were to be found, namely, one section from kidney and one from liver. I have also carefully avoided including in this list the group of fuchsinophilic cell inclusions so frequent in tumor cells and necrobiotic cells of all types.[4]

There has been much discussion as to the occurrence of intravascular fuchsin bodies. Several observers have described forms resembling hyaline thrombi, but whether or not these bodies are to be classed as fuchsin bodies, and in some cases, whether or not they are really intracapillary are disputed questions. This type of body is shown best in gastro-intestinal material; gastric polyps furnish particularly good examples. In sections from such conditions, I have noted large hyaline masses completely filling capillaries which are possibly true hyaline thrombi; again, hyaline masses similarly situated showed lines marking off individual bodies of various sizes composing the mass, and finally, clusters of perfectly discrete fuchsin bodies, typical in every respect, filled small capillary vessels. These capillaries are frequently so situated that their contents may have been subjected to the post-mortem action of certain ferments, notably gastric juice; this observation suggests the possibilities that these bodies are artifacts.

As has been pointed out, the areas in which fuchsin bodies occur most numerously are granulation tissue areas, rich in plasma cells, and they are equally rich in newly formed capillaries. Cross sections of such capillaries frequently present the "signet-ring" appearance. When such capillaries contain hyaline thrombi or

[4] The cellular and nuclear inclusions referred to were included in the Russell (10) description and are still considered by many as belonging to the general class of "fuchsin bodies." I have omitted them to avoid discussing the type of body to which I refer in this connection.

fuchsin bodies, they are very difficult to distinguish from plasma cells containing fuchsin bodies and a nucleus crowded far eccentrically. I have observed even large vessels containing numerous fuchsin bodies. This has been noticed, particularly after alcohol fixation, where one of two conditions existed: (1) either the red blood corpuscles were laked and showed only as shadows; or (2) more frequently, no red blood corpuscles could be made out as the contents of the vessel were more or less fused and shrunken to one side of the vessel wall, while the free edge of the mass showed pseudopodia and nodular projections, some of which were very bulbous at the extremity and suggested constriction; in the open space were numerous perfectly typical fuchsin bodies. The tissue immediately about such areas stains strongly with the same stain as the fuchsin bodies, while at a little distance, the field is filled with typical intra- and extracellular fuchsin bodies. This appearance marks the smaller vessels and their course can frequently be traced through the tissues by this halo of fuchsin bodies, and what I call "blood staining" of the tissues.

If, as has been claimed, the fuchsin body is the result of a degenerative process in the plasma cell, these cells, in areas rich in fuchsin bodies, should show some evidence of degeneration other than the mere presence of fuchsin bodies, unless plasma cell degeneration is always indicatd by such formations. This we know is not the case. It has been my experience that the plasma cells in fuchsin body areas are remarkably well preserved, as a rule, while in areas showing degenerative changes and necrobiotic cells, fuchsin bodies are exceedingly rare. For example, necrotic areas of tumors and infarcts or abscesses seldom show typical fuchsin bodies, while the active periphery of such areas may show them in abundance.

While there is thus no relation between fuchsin bodies and degenerations, and while perusal of the literature shows that conflicting results have been obtained by those who have employed an iron reaction (3, 11), the distribution of fuchsin bodies in various tissues shows a very definite relation to blood vessels and to red blood corpuscles and to pigments derived from them. The presence of some type of hematogenous pigment is practically constant, though relationship between such pigment and the fuchsin bodies is

not always traceable. Hematin occurs so commonly in all sections that, as a rule, its presence is of no significance. There are cases, however, in which it is so closely associated with fuchsin bodies, that its presence can not be disregarded. In the case of chorio-epithelioma, previously referred to, I have been observing the progress of certain changes with reference to hematin and fuchsin bodies. The specimen was fixed in alcohol and all red blood corpuscles were laked. When first studied, there was an abundance of typical fuchsin bodies and crystalline forms, especially within and about some large vascular spaces; there were very few hematin masses. At present, after two years preservation in alcohol, there are fewer typical fuchsin bodies, and the areas previously occupied by them are filled with irregular fuchsinophilic masses and masses of hematin. Likewise, many of the fuchsin bodies show clumps of hematin about them and imbedded in them while some of the hematin masses show the form and grouping of fuchsin bodies.

Hematoidin and hemosiderin have both been observed in association with fuchsin bodies. Neither of these have I found to be constant though both are frequent findings, particularly hemosiderin.

The first point I wish to emphasize is the distribution of fuchsin bodies in chronic abscess formation. This condition is well illustrated by one specimen in my series from an abscess of the broad ligament.

The history of the case indicated a duration of eight years. The material was fixed in formalin. In describing the condition found in the wall of this abscess, we might regard the section as divided into three zones, from within outward: (1) zone of pus, (2) granulation tissue rich in plasma cells, (3) zone of hyaline connective tissue with a few foci of plasma cells. The zone of pus showed only an occasional fuchsin body, or cluster, and exceedingly few red blood corpuscles. The granulation tissue zone was crowded with fuchsin bodies of all types. There were large solitary bodies, clusters of large and small bodies, and both extra- and intracellular bodies of the granular type. There were also a number of hemaglobin-stained plasma cells. Numbers of these bodies showed atypical staining. Very few extravascular red blood corpuscles could be distinguished. Corpuscles in the vessels, though staining sharply, showed no distinct outlines but a decided tendency to fusion. Cross sections of some capillaries contained hyaline thrombi. There was an unmistakable parallelism between distribution of capillaries and fuchsin bodies. At the border line between Zones 2 and 3, fuchsin bodies were relatively few, except in vascular foci. Again in the plasma cell areas of Zone 3, there were a few fuchsin bodies. As the fuchsin bodies decrease from within outward, hematoidin and hemosi-

derin increased. The outer foci of plasma cells showed rich intra- and extracellular deposits of these pigments. A few plasma cells were observed containing both fuchsin body and pigment. The red blood corpuscles in the outer zone were more discrete.

The points to be particularly noted here are:

1. Absence of fuchsin bodies in areas of pus formation.

2. Absence of fuchsin bodies from inactive scar tissue.

3. Presence of large numbers of fuchsin bodies in active granulation tissue showing: (*a*) intravascular autolysis of red blood corpuscles; (*b*) absence of demonstrable extravascular red blood corpuscles; (*c*) parallelism in distribution of capillaries and fuchsin bodies.

4. Association of fuchsin bodies with hematogenous pigment.

My series of vascular lesions, including hemorrhages, thrombi, and infarcts, have shown some important pictures. Areas of hemorrhage, when large, or when fresh, are usually negative, except about the periphery. Smaller areas, showing organization, will show cells containing red blood corpuscles, some typical fuchsin bodies, as a rule, and cells with fuchsinophilic granules as well as cells containing variable amounts of pigment.

In a ruptured tubal pregnancy in which the walls of the tube contained diffuse extravasation of blood, the following condition was found.

There had evidently been oozing of blood for some time, as there was a well-marked phagocytic and plasma cell reaction with abundant production of iron-containing pigment. Most of the red blood corpuscles were well preserved. There were only a few typical fuchsin bodies, though atypical forms were numerous. Many of the large mononuclear and plasma cells showed fuchsinophilic granules. Cells of identical character, containing finely granular hemosiderin, were still more numerous. In addition, there were clusters of globular pigment masses and, associated with these, globular bodies of various sizes taking different degrees of the fuchsin stain.

A section from the wall of an old aneurysm, with a large thrombus mass, showed a quite similar condition.

The wall of the aneurysm was infiltrated with lymphocytes and plasma cells. Portions of the wall near the thrombus showed numerous fuchsin bodies, mostly irregular in form. Further out, these bodies gradually decreased and were replaced by hemosiderin. The pigment in the inner layers (as were the fuchsin bodies) was largely within the plasma cells, while further out it was deposited in irregular masses. This was the first case in which I was able to demonstrate both fuchsin bodies and hemosiderin in the same cell.

Fuchsin bodies have been but little studied in such conditions as the above and I should like to point out in this condition:

1. The occurrence of fuchsin bodies in or about areas of red blood corpuscle destruction.

2. The relation between the distribution of hemosiderin and fuchsin bodies, with reference to thrombus or area of hemorrhage.

3. The identity in the cell types containing fuchsin body and pigment, particularly plasma cells.

It is well to note here an exception to the staining results mentioned with the method I have suggested. The canalized fibrin in material I have used, though formalin fixed, stained intensely with fuchsin. There were, likewise, minute and large globular masses of highly refractile nature within the meshes of the fibrin that also took an intense fuchsin stain. I consider it not improbable that the latter were of the same nature as true fuchsin bodies, and that the fibrin was permeated with hemoglobin, as numbers of the red blood corpuscles were laked and did not stain.

The intimate association of fuchsin bodies with plasma cells led me to study lymphoid tissues. Unfortunately, most of the tissue available for this study was fixed in Zenker's fluid and the staining method relied on for other observations could not be employed. Hematoxylin and eosin and van Gieson's stains showed fuchsin bodies to be relatively frequent in these tissues. I have demonstrated them in acute and chronic lymphadenitis, lymphatic hyperplasia, and in glands of tuberculosis, typhoid, and Hodgkin's disease. In one case of tuberculosis, formalin fixed and stained as usual, there were a large number of medium-sized bodies almost entirely confined to cells of the plasma cell group. Cells of the same type with fuchsinophilic granules were also numerous. Both types of bodies were irregularly distributed throughout the section. Though fuchsin bodies were demonstrated in numerous sections from the spleen, one specimen from a case of pernicious anemia showed an unusually large number of typical intra- and extracellular bodies. There was a high percentage of "granular cells" in the specimen; many were of a type which might have come from the blood stream in such a condition, though there was an unusually large number of the type of relatively large lymphocytes and plasma

cells with typical cogwheel nucleus. The pigment deposits were comparatively slight. Irregular fuchsinophilic masses and even an occasional typical, globular form could be noted, associated with these deposits of pigment. It is of interest to note that both the kidney and liver from this case showed fuchsin bodies in the tubular epithelium and liver cells, respectively. They were mostly small, granular forms but some larger than a red blood cell.

The observations on lymphoid tissues from the gastro-intestinal tract agree closely with the findings in the lymph gland and spleen. Cells containing red blood corpuscles have been carefully excluded in all these specimens. Three specimens from the liver, besides the one above noted, showed fuchsin bodies in liver cells. Two of these were cancer metastases with abundant pigment deposits, the third, biliary cirrhosis. This section also showed pigmentation.

My study of these tissues has shed but little light upon the subject. The only points to be made out with certainty are as follows:

1. Fuchsin bodies are relatively frequent in lymphoid tissues in a variety of conditions.

2. Fuchsinophilic granular cells are numerous, although the conditions under which they occur make it difficult to distinguish them from true eosinophiles.

3. There is evidence of association with pigment production.

4. Lastly, fuchsin bodies occur in tubular epithelium and liver parenchyma in association with pigmentation.

As previously indicated, fuchsin bodies frequently occur in areas which have been subjected to the post-mortem action of certain ferments, such as the gastric juice, and wherever the red blood corpuscles show evidence of such action, the number of fuchsin bodies is correspondingly increased. Further, I have noted carefully the effects of fixation on the number and type of fuchsin bodies and have observed the associated changes in other elements which might give some clue as to the origin of fuchsin bodies. The results are briefly that within certain limits the number and type of fuchsin bodies may be decidedly influenced by fixation. As a rule, the number of typical bodies is inversely proportional and granular forms are influenced to a less extent. Fixation by

dilute alcohol, or by a small amount of stronger alcohol, or by fluids containing bichromate gives the largest number of typical fuchsin bodies and causes laking of the red blood corpuscles, or autolytic changes with fusion of the corpuscular mass, where larger pieces of tissue are used. After rapid fixation with 10 per cent. formalin there are few typical fuchsin bodies, but, instead, forms resembling fragmented red blood corpuscles and many granular forms. The degree of preservation of nuclei of cells has a decided relation to fuchsinophilic cellular or nuclear inclusions; otherwise the changes in red blood corpuscles are the only constant tissue changes that seem to have any relation to the number and type of fuchsin bodies present.

Thus far, I have attempted to show that the fuchsin body represents a type subject to variation in morphology, and staining reactions, and that in addition to the forms generally recognized as typical, there are, at least, two varieties which might be regarded as atypical: those atypical in morphology, and those atypical in staining reactions.

This broader conception of the so-called fuchsin body seems essential to any explanation of the phenomena observed in a comprehensive study of these bodies. Further, such a conception clears up a great many of the seeming contradictions which exist in the literature and are undoubtedly traceable to differences in type of fuchsin body studied by various workers.

The occurrence of fuchsin bodies in cells other than plasma cells, the occurrence in and near blood vessels, the association with hematogenous pigments, and the identity of staining reactions of fuchsin bodies and red blood corpuscles are points deserving especial emphasis.

The cellular and vascular relations of fuchsin bodies have been the subjects of greatest controversy. That no single type of tissue cell, or, of necessity, any tissue cell whatsoever can be held accountable for the production of these bodies, is evident to any one who will extend the material for his investigation over a sufficiently wide field. That cells of the plasma cell type are most commonly associated with fuchsin bodies is quite evident, but it is equally evident that fuchsin bodies occur in areas free from plasma cells and even

within blood vessels in the absence of any cell save those from the blood stream.

The staining reactions are, to say the least, strongly suggestive of a relation to red blood corpuscles. Every stain that I have employed has shown an identity in staining reaction between fuchsin bodies and red blood corpuscles. The only exception is where the red blood corpuscles have been laked and do not stain at all. Eosinophile granules are the only other structures sharing this identity of staining.

The association with hematogenous pigments, particularly where fuchsin bodies and iron-containing pigment are demonstrable in the same cell, furnishes some of the strongest evidence of a possible origin of fuchsin bodies from red blood corpuscles. While thus the relation of fuchsin bodies to red blood corpuscles is apparent, certain features (alcohol fixation) suggest the possibility that these bodies are artifacts. That they are artifacts only in part, I feel sure, as fuchsin bodies are present in the tissues even after the best possible fixation and where the red blood cells show perfect fixation. According to my observations, as I have previously pointed out, the bodies in such specimens are predominantly types which are granular or resemble fragments of red blood corpuscles. It appears that these must constitute the fundamental types, and that other types found in sections are derived from these. Mere alteration in form or coalescence of granules might give rise to the larger and oval or rounded forms. This tendency would be aided by slow fixation, as in alcohol. The theory of coalescence of granules is in harmony with the views of Schridde (12) and Fabian (2), though I believe my interpretation of the nature of these granules would hardly coincide with theirs (13). While, as I have before stated, these granules are often indistinguishable from eosinophile granules, yet I believe they are undoubtedly derived from the red blood corpuscles. It might be recalled that the conditions in which fuchsin bodies occur are, in the main, conditions in which there is local or general destruction of red blood corpuscles.

It seems certain that hemoglobin is one factor concerned in fuchsin body formation. Whether this is the sole factor, or in what form it exists, will require further work to determine abso-

lutely. In those instances in which the fuchsin bodies have a definite crystalline form they correspond closely with the parahemoglobin of Nencki (9). As previously shown for intravascular fuchsin bodies, autolysis with fusion of red blood corpuscles may give rise to fuchsin bodies, in which case the entire corpuscle is concerned in such formations. I have reason to believe that in some cases the pigment nucleus has been split off, and the fuchsin body represents mainly or solely the "globin." This material might enter plasma cells and other cells by diffuse staining of cells or simple diffusion. Plasma cells stained by hemoglobin are not uncommon, in fact, plasma cells seem to have an especial affinity for hemoglobin and I consider this of significance. It will be noted that the hematogenous pigments are found abundantly in the same classes of cells in tumors, chronic inflammatory tissues, spleen, lymph glands and other organs.

Our knowledge of hemosiderin, its mode of formation, its chemical and physical constitution, is too slight to permit of any positive assumption on these points. According to Neumann (19), it may be formed both intra- and extracellularly, but only under the influence of living cells. But little is known concerning the possible transitional stages from hemoglobin to hemosiderin. However, in such organs as the spleen, where abundant deposits of hemosiderin are present, fragments of red blood corpuscles, granules and droplets of blood coloring matter are very common in association with this pigment. These "hematogenous droplets" show varying degrees of affinity for such stains as eosin, as well as gradations in iron-reaction. The fuchsin body in the plasma cell seems to bear an identical relation to the hematogenous pigments in these cells; this relation is particularly obvious when both fuchsin body and pigment can be observed in the same cell. As I have pointed out, there is a striking parallelism in certain instances between the distribution of fuchsin bodies and hemosiderin.

From this and similar evidence it seems probable that the fuchsin body with its atypical groups may represent intermediate products in the formation of hemosiderin from hemoglobin by plasma cells and allied cell groups. It can not be claimed that all fuchsin bodies represent factors in such a process. Undoubtedly, a number of

such bodies are artifacts due to post-mortem action of certain enzymes or to mode of fixation.

Finally, I may seem to hold the view that all fuchsin bodies have their origin from red blood corpuscles. This, however, does not represent my opinion. While believing firmly that fuchsin bodies may and do arise from red blood corpuscles, I have been unable to convince myself that this is, or is not, the sole source of such bodies. Changes in nuclear material, which I have purposely avoided discussing in this paper, seem to demand closer study than has been accorded them in this connection, and I have been unable to exclude such changes as a second possible source of fuchsin bodies.

SUMMARY.

In summarizing the results of this work I feel justified in drawing the following conclusions:

1. The fuchsin body represents a type of body subject to wide variations in morphology and in staining reaction.

2. Though more frequently associated with plasma cells than with any other cell type, these bodies may and do occur in a great variety of cells.

3. Further, certain sections show unmistakable fuchsin bodies within blood vessels; some of these bodies are true hyaline thrombi, while others are due to autolytic changes in the red blood corpuscles.

4. Fuchsin bodies are most numerous in granulation tissue and lymphoid areas. In the former, they follow closely the distribution of capillaries and show a parallel with the changes in the extra- and intravascular red blood corpuscles.

5. The number and type of fuchsin bodies in tissues can be influenced by methods of fixation, the number of typical bodies being inversely proportional to the rapidity and degree of fixation of the red blood cells.

6. The type of body found after the best fixation is not, as a rule, what is considered the typical form, but an irregular fragmentary type or the fuchsinophilic granular form.

7. The identity in staining reaction, the constant association with changes in red blood corpuscles, the relation to distribution of capillaries in granulation tissues, the occurrence in hemorrhagic lesions

associated with pigment, the intravascular occurrence, and, finally, the close relation to pigment deposits, all indicate conclusively that fuchsin bodies arise from red blood corpuscles.

8. No theory of origin from plasma cells, or other granular cells, offers an adequate explanation for the origin of fuchsin bodies, nor can any theory prove adequate which does not take into account the red blood corpuscles.

9. The peculiar association of plasma cells with fuchsin bodies and hemosiderin seems explainable on the assumption that the plasma cell in such instances is the active factor in a metabolic process, the fuchsin body representing a stage in the metabolism of hemoglobin by an intracellular enzyme, and hemosiderin, one of the products of the process.

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A STUDY OF AN EXTREMELY PURE PREPARATION OF RICIN.[1]

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In September, 1905, Osborne, Mendel and Harris (1) published a paper entitled, "A Study of the Proteins of the Castor Bean, with Special Reference to the Isolation of Ricin." In their study they found that the toxic substance known as ricin was associated with the coagulable albumin of the castor bean, and that of this protein 0.0005 milligrams per kilo of body weight injected into a rabbit caused death on the seventh day, and a dose of 0.0032 milligrams per kilo injected into a guinea pig caused death in seven days. With their preparation (No. 15), the susceptibility of the rabbit and the cat were about equal, 0.1 milligrams per kilo causing death in two to three days in these two animals. In the guinea pig and dog, 0.5 milligrams per kilo caused death within forty-eight hours. They did not test in any more detail this preparation, which was about one hundred times more toxic than that of Cushny, who had up to this time prepared the most potent ricin. I shall not go into a further discussion of the historical aspect of this subject, but shall proceed to detail my results.

Preparation.—In the preparation of the ricin used in this work I followed exactly the method detailed by Osborne, Mendel and Harris. Although I followed their method in every step, I did not get the proportionate yield of ricin that they obtained, probably owing to their greater experience in the isolation of proteids. Roughly stated, the method consists of extracting the ground beans for forty-eight hours with 10 per cent. sodium chloride solution, two liters of solution to the kilo of meal.[2] This is filtered and

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[2] All the oil has previously been removed from the ground beans by extraction with ether and pressure and then a further grinding.

then dialyzed for four or five days. It is again filtered and the precipitated globulin removed. To the filtrate is then added enough ammonium sulphate to bring it to a 45 per cent. concentration. After standing the precipitate is filtered off and dissolved in water, after which is added an equal quantity of the saturated ammonium sulphate solution. It is then allowed to stand over night and again the precipitate is removed by filtration.[3] The dried precipitate then contains less ammonium sulphate than it otherwise would. After this, the partially dried precipitate is dissolved in water, and enough saturated ammonium sulphate solution is added to bring it to a 33 per cent. concentration. When the precipitate that has been formed by this concentration of ammonium sulphate has settled, it is collected on a filter and then dissolved in water and dialyzed, first for seven days against running tap water and then against distilled water for another week. It is then carefully filtered and the filtrate evaporated as rapidly as possible to dryness *in vacuo*. The coagulable albumin so isolated is the most toxic fraction to be obtained from the proteins of the castor bean. Another fraction of coagulable albumin may be obtained by bringing the filtrate of the 33 per cent. ammonium sulphate concentration to a 50 per cent. concentration and this is also toxic but less so than the precipitate obtained from the 33 per cent. concentration, as the precipitate from the 50 per cent. concentration contains a greater quantity of proteose. All of the results detailed in this paper were obtained with the albumin precipitated by a 33 per cent. concentration of ammonium sulphate. Osborne, Mendel and Harris found in their preparation of this fraction coagulable albumin, 70.64, and proteose, 29.36.

To determine the amount of coagulable protein and proteose in the preparation used in this work, 0.827 gram, calculated as ash free,[4] were taken and dissolved in 10 per cent. sodium chloride solution. It was heated on a water bath for one and a half hours at 94° to 96° C. The coagulum was filtered off, washed, dried and weighed. It contained 0.524 gram, leaving .203 gram of proteose which

[3] These protein precipitates are best deprived of the excess fluid and salts by placing them between large quantities of filter paper and applying a gradually increasing pressure.

[4] Ash = 5.16 per cent.

gave the following result of percentages: coagulable albumin, 75.46; proteose, 24.54. It will be seen that this agrees fairly well with the figures obtained by Osborne, Mendel and Harris, except that the coagulable proteid is slightly higher.

The Toxicity of the Preparation.—Osborne, Mendel and Harris found that the minimal lethal dose of their most toxic preparation for various animals was as follows: for rabbits, 0.0005 milligram per kilo; for guinea pigs, 0.0032 milligram per kilo; for dogs, 0.5 milligram per kilo (death in forty-eight hours); for cats, .1 milligram per kilo.

The minimal lethal dose of the preparation under discussion was as follows: for rabbits, 0.0001 milligram per kilo; for guinea pigs, 0.0008 milligram per kilo; for dogs, 0.0006 milligram per kilo, for cats, 0.0002 milligram per kilo; for goats, 0.003 milligram per kilo (death in three days).

In the case of the goat this dose caused death on the third day. The autopsy findings in these various animals were similar. These doses are based on intramuscular inoculation. Intravenous injection gave a shorter incubation period, but no great difference in the minimal lethal dose. The time of death could be gauged within rather narrow limits by varying the size of the dose. In rabbits, 10 milligrams per kilo caused death in five to six hours. One hundred milligrams gave exactly the same result, showing that the minimal incubation period is between five and six hours. The pathological lesions were never as marked in these cases of short incubation periods as in those animals dying from the third to the sixth day. Death could be regularly produced up to the sixth day, but after this period, individual variations in the susceptibility of the animals became an extremely disturbing factor. Lesions caused by these extremely potent preparations are identical with those produced by the ordinary commercial preparations.

In 180 animals injected with fatal doses of ricin, the results were as follows:

The autopsy findings were intense hemorrhagic edema at the site of inoculation. The peritoneal cavity always contained a quantity of bloody fluid, varying in amount from 50 cubic centimeters to as much as 200 cubic centimeters. Nine per cent. of the ani-

mals showed the presence of a bloody fluid in one or both pleurae. Three per cent. showed the presence of this fluid in the pericardium. The heart and lungs were normal. About 5 per cent. of the animals showed a localized hemorrhagic area in the thymus; 2 per cent. in the thyroid gland; 15 per cent. in the pancreas; 45 per cent. in the adrenals. The pyloric portion of the stomach and the small intestine showed these lesions in every case. In the small intestines the lesions were more numerous in the duodenum. The lymph nodes of the peritoneal cavity were always enlarged and softened. The liver, as a rule, showed marked congestion and many areas of focal necrosis. The spleen was large, soft, and extremely engorged with blood. The microscopical examination of specimens from these various tissues showed exactly the same picture that Flexner (2) has described. The post-mortem picture is in some particulars similar to that caused by diphtheria toxin, especially the lesions at the site of injection in the adrenals and stomach.

The Hemagglutinin.—This function of the preparation was tested against the red blood cells of horses, rabbits, guinea pigs and dogs. One cubic centimeter of a 1 per cent. suspension of well-washed red blood cells, plus the ricin in one cubic centimeter of .9 per cent. sodium chloride solution. The amount of ricin required to bring about complete agglutination of the above quantity of red blood cells varied between 0.002 and 0.005 for all four species of red blood cells, showing that there was not any greater variation between the minimal agglutinal doses than between the minimal lethal doses.

Keeping Qualities.—At the end of two years and a half this preparation was tested for its toxicity in rabbits, dogs, and guinea pigs, and it was found to be absolutely inert, one-half gram producing no effect in a rabbit. On the other hand, the minimal agglutinating dose was only slightly raised. It now required between 0.005 to 0.009 gram to bring about complete agglutination of one cubic centimeter of a 1 per cent. red blood cell suspension.

Effects of Electric Current.—One gram of this protein preparation was dissolved in twenty-five cubic centimeters of distilled water and placed in a cell, similar to the one described by Field

and Teague (3), with agar-filled tubes as electrodes. A direct current was passed through this solution for five hours, the strength of the current being one hundred and ten volts, and from three to eight milliamperes. After a very short time a white flocculent precipitate appeared about the anode agar. At the end of this time, as no more precipitate was seen to be forming, this precipitate was collected on filter paper and washed thoroughly in distilled water, in which it was insoluble. It was then treated with water containing a slight trace of sodium carbonate, in which it was readily soluble. This preparation was far more active as a hemagglutinin than the original preparation. On the other hand, the minimal lethal dose was markedly decreased. The figures were as follows: .0001 milligram agglutinating dose, as against 0.002 to .005. The minimal lethal dose for rabbits was .002 milligram, as against 0.0001. The filtrate showed a very marked loss in agglutinating value per milligram of protein, but it also showed a loss in the minimal lethal dose as well.

CONCLUSIONS.

1. By following the method of Osborne, Mendel and Harris, we can obtain an extremely potent toxin from the castor bean.

2. It would appear, as a result of testing this preparation at the end of two and a half years, that the agglutinating function and the toxic function are two distinct properties.

3. This result is also borne out by the behavior under the electric current; either we are dealing with two different substances or else with a single substance with two distinct toxiphore groups, one of which is stable and the other labile.

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NEUROCYTOMA OR NEUROBLASTOMA, A KIND OF TUMOR NOT GENERALLY RECOGNIZED.[1]

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PLATES XLIV–XLVI.

The object of this paper is to call attention to a group of tumors, the nature of which has hitherto escaped general recognition, and to point out their distinguishing characteristics. These tumors are rich in cells which may vary much in size and shape. They may be pervaded by connective tissue by which the cells are arranged in more or less definite alveoli. Blood vessels accompanied by connective tissue are also present. The essential cells of the tumor are considered to be more or less undifferentiated nerve cells or neurocytes or neuroblasts, and hence the names neurocytoma and neuroblastoma. They are considered to be neurocytes or neuroblasts for the following reasons:

1. The cells, at least in places, are associated with delicate fibrils, often of considerable length, which do not stain like neuroglia, collagen or fibroglia fibrils by Mallory's methods, and which are like the fibrils occurring in the "anlage" of the sympathetic nervous system.

2. The cells associated with these fibrils have the same morphology as the cells from which the sympathetic nervous system and the medulla of the adrenal develop, and which are regarded by embryologists as arising from migrated primitive nerve cells. They are generally small, with round nuclei rich in chromatin, and have a relatively small or imperceptible amount of cytoplasm. Some of them may be of piroform shape and their cytoplasm may be prolonged into filamentous processes like the fibrils referred to above.

3. The cells and fibrils are arranged in places in ways more or

[1] Received for publication May 19, 1910.

less like the ways in which the neurocytes and fibrils are arranged, both in the sympathetic nervous system at certain periods of its development and in the "anlage" of the adrenal.

Thus the fibrils may be arranged parallel to one another in bundles which are intimately associated with masses of the cells, as are the fibrils with the neurocytes in the "anlage" of the adrenal of a human fetus in the fourth or fifth month (compare Fig. 11 with Fig. 12). Moreover, the bundles of fibrils may extend into masses of the tumor cells as do the fibrils into the mass of neurocytes constituting the "anlage" of a sympathetic ganglion. The connective tissue stroma may more or less completely demarcate masses of cells with their associated bundles of fibrils and such masses of cells and fibrils may be very like the sympathetic ganglia of a human embryo of about the sixth week. This will be clear by comparison of Figs. 1, 2, 9 and 10 with Figs. 3 and 8.

Another mode of arrangement of the cells and fibrils is that of ball-like aggregations about forty micra or more in diameter, enclosing a small mesh-work of fibrils and of filamentous processes of the cells which do not stain like neuroglia or connective tissue fibrils by Mallory's specific staining methods. If the section passes through the equatorial plane of one of these ball-like cell masses, it appears as a circular mass formed of one or more rows of cells surrounding a central mass of fibrils and filamentous cell processes. These have been called "rosettes." These ball-like aggregations are like the ball-like aggregations of neurocytes, both in morphology and staining reactions, which occur in the sympathetic ganglia and in the adrenal as well as in the connective tissue about these structures at certain periods of fetal life (compare Fig. 4 with Figs. 5, 6 and 7).

The characteristic fibrils of these tumors may be present only in restricted areas of the tumor tissue. By degeneration or post-mortem changes they may become more or less transformed into hyaline or granular material and thus escape recognition. This is especially true of the fibrils in the central parts of the "balls" of cells, both of the tumor and of the fetus.

Thus two characteristic modes of arrangement of the cells and fibrils may be distinguished, namely, the arrangement of the fibrils

in bundles associated with masses of the cells and the ball-like formations above described. Both modes of arrangement are not found in every case.

The cases of tumor formation of the nature here described, which I have personally observed or have been able to recognize in the literature, may be summarized as follows:

CASE I. Reported by N. Dalton (1). Tumors of liver and left adrenal in a male child, 6 weeks old. From the description and drawing of the microscopical appearances of the adrenal tumor, it is plain that the characteristic ball-like arrangement of the cells and fibrils were present. Thus the author states that the small cells "are mingled with a peculiar, finely granular and, in places, perhaps slightly fibrillated substance." "In some places the round cells are scattered uniformly through this material. In others, they are arranged around the borders of a small mass of it, something like the nuclei of a giant cell." "This material is stained deeply by picro-carmine and may possibly be the degenerated reticulum of a lymphomatous growth."

CASE II. Reported by F. Marchand (2). Tumor of right adrenal in a female child, 9 months old. The tumor is described as consisting of a tissue like that of the "anlage" of a sympathetic ganglion, and drawings accompanying the paper confirm the description. This is the first time that the true nature of a tumor of this kind was recognized.

CASE III. Reported by S. Amberg (3). Tumors of both adrenals and of liver in a female child, 2 months old. The author's description leaves little doubt that the tumor tissue showed the ball-like aggregations of tumor cells with hyaline transformation of the central fibrils, but their significance was apparently not recognized. He noted, however, a striking resemblance of the tumor cells to the cells in the medulla of the adrenal of a six month fetus.

CASE IV. Reported by H. Küster (4). Tumors of both adrenals and of liver in a child 14 weeks old, sex not given.

CASE V. Reported by H. Küster (4). Tumor of adrenal in an adult male. The tumors in this and the preceding case contained the characteristic ball-like aggregations of the cells and fibrils. They were regarded by the reporter and by Prof. Ribbert as closely allied to glioma. J. Wiesel (5), however, later pointed out that in his opinion the tumors were composed of undifferentiated cells of the sympathetic nervous system, which he had shown to invade regularly the "anlage" of the adrenal and to give rise to the chromatin cells and ganglion cells of the medulla of that organ.

CASE VI. Reported by O. Richards (6). Tumors of the adrenal and liver in a male child, 2 weeks old. The tumor was described as a small, round cell sarcoma. The author states that "In part the cells of the tumor are arranged around the edges of small masses of homogeneous substance of uncertain nature." From this description it seems right to infer that the tumor showed the characteristic ball-like arrangement of the cells enclosing hyaline degenerated central fibrils.

CASE VII. Reported by A. Lapointe and P. Lecène (7). Tumors of right adrenal, retro-peritoneal tissue and mediastinum in a female child, 19 months old.

The tumor was regarded by the reporters as a glioma. This diagnosis was also made by Prof. Ribbert, who examined some of their preparations. Their drawings and description of the cells and fibrils show beyond reasonable doubt that the tumors belong to the group here described.

CASE VIII. Reported by W. Tileston and S. B. Wolbach (8). Tumors of right adrenal and in cranium, involving adjacent soft tissue and lymph nodes in a male child, 16 months old. For material from this case I am indebted to Dr. Wolbach. The ball-like arrangement of the cells and fibrils is a prominent feature of the tumor tissue which was considered to be of a lymphomatous nature (Fig. 4).

CASE IX. Tumors of both adrenals in a still-born male child. I am indebted to Dr. S. R. Haythorne for preparations from this case and for permission to refer to it here. The microscopical characters of the tumor tissue are shown in Fig. 11 (compare Fig. 12).

CASE X. Large tumor in hilum of lung and multiple tumors in liver in a male adult. I am indebted to Dr. R. Kinnicutt for this case. The characteristic fibrils are found only in the tumor of the lung and only in places in it. The most characteristic area is shown in Fig. 2. The cells of this tumor are very variable in size, some of them being very large. At one place they are of columnar shape and disposed radially about blood vessels or about lumen-like spaces.

CASE XI. Tumor of the cerebellum. This was removed at operation by Dr. Harvey W. Cushing, to whom I am indebted for permission to refer to it here. I am also indebted to Dr. F. B. Mallory for calling it to my attention and for the opportunity of examining microscopical preparations from it. The tumor tissue shows the characteristic ball-like arrangement of cells and fibrils in places.

CASE XII. Multiple tumors of skull, mediastina, retro-peritoneal tissue with involvement of pelvis, liver, and some other situations in a female child, 16 months old. Autopsy No. 227, Massachusetts General Hospital. The tumor tissue in this case presents in places the appearance of being composed of aggregations of more or less atypical embryonic or fetal sympathetic ganglia bound together by connective tissue stroma (Figs. 1, 9 and 10). The tumor tissue in the skull contains considerable bone in the stroma and the masses of cells often enclose or border upon cystic cavities.

I have also seen microscopical preparations of two other cases of small-cell tumor of the head, which clearly show the ball-like arrangement of the cells and fibrils.

The occurrence of these tumors in such a variety of situations is accounted for not only by metastasis but also by the generally accepted teaching that undifferentiated nerve cells migrate from the embryonic central nervous system to form the nerves and ganglia as well as the sympathetic system. Therefore, theoretically, these tumors may occur in any region of the body. Their occurrence in the adrenal is explained by the now generally accepted doctrine of the invasion of the "anlage" of the cortex of that organ by undif-

ferentiated cells of the sympathetic nervous system, or so-called formative cells of the sympathetic, to form the cells of the medulla, as was first clearly shown by J. Wiesel (9). This doctrine also accounts for the apparent medullary origin of some of the tumors. The involvement of the adrenal in such a large proportion of the cases, with simultaneous involvement of the liver or of the cranium, in some cases, strongly supports the belief that other small-cell tumors of the adrenal that have been reported in man and the ox, and other cases of simultaneous small-cell tumor involvement of the adrenal and liver or cranium among those collected and reported by R. Hutchinson (11), by W. Pepper (10) and by W. Tileston and S. B. Wolbach (8), are also cases of neurocytoma in which the characteristic fibrils have not developed or have been overlooked. Essentially the same view for the simultaneous sarcomas of the adrenal and liver was expressed by R. Kretz (12) some years ago, when he suggested that such tumors came from the formative cells of the sympathetic system.

Moreover, it seems reasonable to believe that certain other small-cell tumors without the characteristic fibrils, occurring in various situations, are also of this nature.

The fact that five of the new cases have been recognized within a year seems to show that neurocytoma is not a very rare tumor and that tumors of this kind will be found to constitute an important group among the tumors now called glioma, sarcoma, lymphoma or lymphosarcoma.

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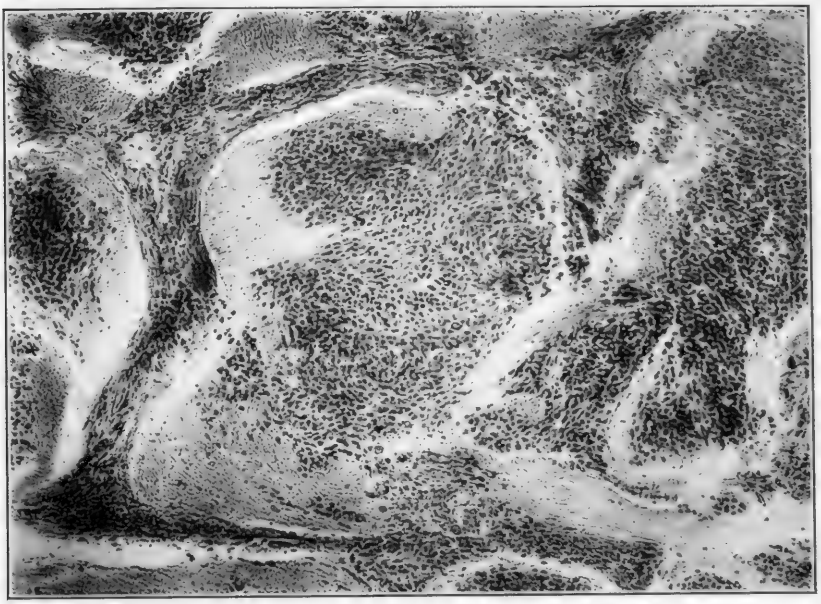

FIG. 1.

FIG. 2.

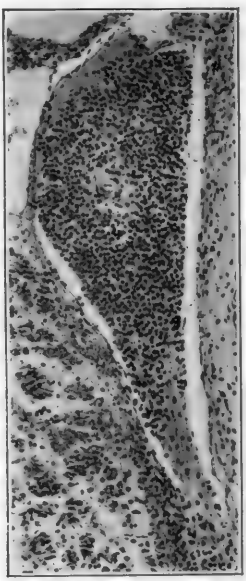

FIG. 3.

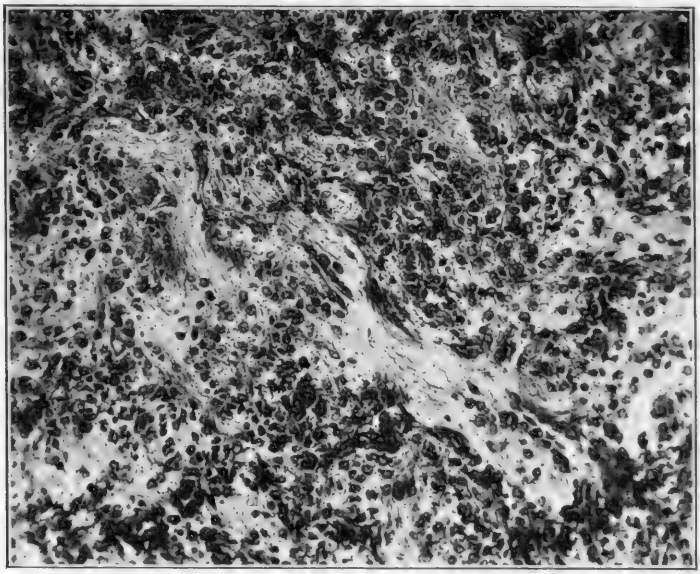

FIG. 11.

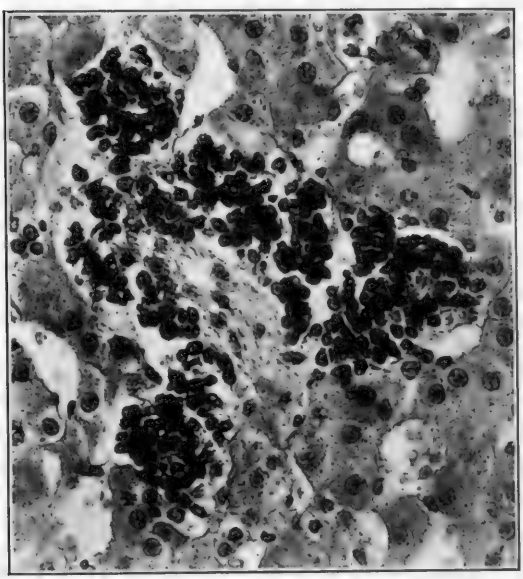

FIG. 12.

EXPLANATION OF PLATES.[2]

PLATE XLIV.

FIG. 1. A characteristic area in the metastasis of a tumor in a lymph node of Case XII, showing the arrangement of the fibrils in bundles associated with masses of small cells.

FIG. 2. A characteristic area in the tumor of the lung of Case X, showing the fibrils arranged in bundles as in the embryonic sympathetic ganglion.

FIG. 3. "Anlage" of a sympathetic ganglion of a human embryo of about the sixth week, showing the arrangement of the cells and fibrils.

PLATE XLV.

FIG. 4. An area in the tumor of Case VII, showing the characteristic ball-like arrangement of the cells. The fibrils in the centers of the cell balls are obscure, probably from degenerative changes in them. Some of the cells are of piroform shape and some of the fibrils may be seen to be processes of the cells.

FIGS. 5, 6 and 7. Showing the ball-like aggregations of neurocytes enclosing the fibrils in their central portions in the "anlage" of the human adrenal or in the connective tissue about it. Fig. 7 shows the piroform shape of the cells and their fibril-like processes.

FIG. 8. "Anlage" of a sympathetic ganglion of a human embryo of about the sixth week showing the arrangement of the cells and fibrils.

FIGS. 9 and 10. Characteristic areas in metastases in lymph nodes of Case XII. Showing the resemblance of some of the masses of cells and bundles of fibrils and associated bundles of fibrils to the "anlage" of a human sympathetic ganglion (compare Fig. 8).

PLATE XLVI.

FIG. 11. Characteristic area in the tumor of the adrenal of Case 9, showing arrangement of the fibrils in bundles (compare Fig. 12).

FIG. 12. A mass of Neurocytes with an associated bundle of fibrils in the "anlage" of the adrenal cortex of a human fetus of about the fifth month.

[2] Photomicrographs by Mr. L. S. Brown, Pathological Laboratory, Massachusetts General Hospital.

SUBCUTANEOUS REACTION OF RABBITS TO HORSE SERUM.[1]

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The object of the following study is to determine the effect of subcutaneous injections of horse serum upon rabbits in order to ascertain whether this procedure can be used as a test of altered reactivity (allergy) or of acquired anaphylaxis. Heretofore most of the experimentation upon anaphylaxis has usually resulted in the death of the animal. Rabbits are known to be less susceptible than guinea pigs to foreign sera, and it was considered possible that local, but quite as definite, phenomena might be obtained in rabbits after repeated inoculations of small quantities of horse serum.

Previous Work.—But few investigators have been interested in this particular inquiry. Arthus (1) in 1903, following the ideas of Richet, first noted the effect of injections of horse serum upon rabbits. He found that the first injections produced no clinical consequences, but that after later injections intense local swellings were produced, and at times definite necrosis. In a few instances sudden death of the animal followed. This result was more frequent after intraperitoneal and intravenous injections. Later von Pirquet and Schick (2) injected rabbits subcutaneously in the ear and produced edema after the second injection, using rather large amounts of serum, one to three cubic centimeters. Whereas Arthus had considered that this production of local reaction de-

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pended on the number of previous injections, von Pirquet and Schick showed that the interval between the first and second injections was the more important factor, and that the local swelling followed even after the second injection, provided that about ten days elapsed between the first and second inoculations. These writers found also that horse serum was specific in its action, that a previous injection of horse serum did not affect subsequent injections of other sera, and that no reaction followed the inoculation of horse serum in animals that had been previously injected with other sera. They found also that animals in which horse serum had been previously injected reacted locally after the injection of rabbit serum from an allergic rabbit.

Later Nicolle (3) produced a local edema in rabbits which had previously received a large amount of serum from an allergic rabbit, by injecting it subsequently with horse serum. Since 1907 nearly all authors have been interested chiefly in the general phenomena of anaphylaxis, and have paid but little attention to the local reaction, and, moreover, most of the work has been done with guinea pigs and not with rabbits. In regard to local reaction on guinea pigs, the observation of Paul Lewis must be mentioned. He showed that in pigs which survived the allergic shock, large areas of edema were often produced.

Description of Test.—In our first experiments twenty rabbits, ten males and ten females, of nearly equal weight, were selected (Table I). Two (Nos. 48 and 37) were reserved as controls. The remaining eighteen rabbits were inoculated in an ear vein with varying amounts of sera as follows (Table I): Nos. 55 and 53, 0.1 cubic centimeter of horse serum;[2] Nos. 51 and 27, 0.1 cubic centimeter of serum and 9.9 cubic centimeters of normal salt solution; Nos. 26 and 78, 0.5 cubic centimeter of serum; Nos. 42 and 71, 0.5 cubic centimeter of serum and 9.5 cubic centimeters of salt solution; Nos. 56 and 30, 1 cubic centimeter of serum; Nos. 4 and 49, 1 cubic centimeter of serum and 9 cubic centimeters of salt solution; Nos. 14 and 35, 5 cubic centimeters of serum; Nos. 34 and 40,

[2] The horse serum used in the first injections was obtained by us from a superannuated but healthy horse. The serum was preserved with 0.5 per cent. carbolic acid and kept on ice. The later experiments were carried out by the serum kindly sent us from the Rockefeller Institute.

5 cubic centimeters of serum and 5 cubic centimeters of salt solution; Nos. 50 and 10 with 10 cubic centimeters of serum each.

On the sixth day after the intravenous injections, one rabbit from each group, ten in all, were inoculated subcutaneously in the ear with 0.1 cubic centimeter of a 10 per cent. solution of horse serum. This produced no local reaction in any of the rabbits. The same ten rabbits were injected with the same amounts of serum every other day for three days. After the third injection, most of the rabbits, as indicated in the table, showed a positive reaction, definite swelling, and redness of the ear at the point of inoculation. The difficulty of inoculating the serum into the scanty subcutaneous tissue of the ear without injury was recognized. It was thought that some of the redness and swelling might be due to mechanical injury, and after the twelfth day of the injection, the tests were made into the carefully shaved skin of the abdomen. As is indicated in the table, local reactions began to appear ten days after the original intravenous injection of serum, and continued with some exceptions noted in the table as long as the animals were tested; that is, the hyper-susceptibility to horse serum developed in the rabbits remained for at least two months and a half. The first test of the second rabbits of each of the ten groups was made on the fourteenth day after the original intravenous injection and consisted in the injection of 0.1 cubic centimeter of a 10 per cent. solution of horse serum into the skin of the abdomen. These second numbers of each group seemed to average about the same number of definite reactions as those which had been injected several times previously. It was, therefore, concluded that the hyper-susceptibility to the serum was the result of the first intravenous injection, and that this hyper-susceptibility developed in most of the rabbits in from ten to fourteen days.

Several interesting individual variations are to be noted. Rabbit No. 48, a control animal, which had received no serum originally, exhibited a suggestive reaction on the third and fifth day after the first subcutaneous injection, and remained definitely positive from the ninth day after this injection. Apparently this animal was unusually susceptible to horse serum and developed its anaphylaxis because of the subcutaneous injection of 0.1 cubic centimeter of a

10 per cent. solution of horse serum. Another rabbit, No. 71, showed complete insusceptibility to horse serum during two months and a half of observation. After the sixteenth day, 0.1 cubic centimeter of undiluted horse serum was used in making the intradermal tests. In general, the reactions seemed a little more constant in those animals that had originally received the larger amounts of serum, though there were exceptions to this observation. On the fifty-sixth day, 0.05 cubic centimeter of horse serum was used intradermally in making the tests. The animals seemed just as susceptible to this smaller amount of serum.

In making the tests it was always endeavored to insert the point of a small hypodermic needle into and not under the skin, and to produce by the injection a small translucent bleb.

To verify these results, a second series of animals, four in number, were each injected intravenously (ear vein) with 5 cubic centimeters of horse serum (Table II). On the sixth day they were tested by an intradermal abdominal injection of 0.1 cubic centimeter of horse serum. None of them reacted. This test was repeated on the fourteenth and sixteenth days with negative results. After a similar test on the nineteenth day, three of the animals reacted positively, but the fourth was not definitely positive until the twenty-seventh day (Table II), and was negative to three subsequent tests but remained constantly positive after the thirty-eighth day. Rabbit No. 48 of the first series, already shown to be susceptible to horse serum, was again injected with this second series as comparison. A sixth rabbit, No. 2, which had received 1 cubic centimeter of horse serum subcutaneously, reacted positively on the thirteenth day to the intradermal injections of 0.1 cubic centimeter of horse serum.

In order to determine whether even smaller intradermal injections of horse serum could not be used to determine the presence of anaphylaxis in rabbits, a third series of rabbits, twelve in number, was carried on (Table III). Of these, two were used as controls, and the others, in groups of two, were injected intravenously with horse serum as follows: first group, controls; second group, 0.1 cubic centimeter; third group, 0.5 cubic centimeter; fourth group, 1 cubic centimeter; fifth group, 2.5 cubic centimeters; sixth group, 5 cubic centimeters. On the sixth day one animal of

TABLE II.

Rabbit.	Day.	Dec. 30	31	Jan. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
6 Wt. 2040 grms.	5. c.c. horse serum intrav.						T^2	−							T^2	−	T^2	−		T^2	+	T^2	+	T^2
66 Wt. 1520 grms.	5 c.c. horse serum intrav.						T^2	−							T^2	−	T^2	−		T^2	+	T^2	+	T^2
3 Wt. 1600 grms.	5 c.c. horse serum intrav.						T^2	−							T^2	−	T^2	−		T^2	−	T^2	−	T^2
9 Wt. 1670 grms.	5 c.c. horse serum intrav.						T^2	−							T^2	−	T^2	−		T^2	+	T^2	+	T^2
48[1]																						T^5	+	T^2
2[2]																							P T^2	+ +

Rabbit.	Day.	Jan. 22	23	24	25	26	27	28	29	30	31	Feb. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		24	25	26	27	28	29	30	31	32	33	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
6 Wt. 2040 grms.	5 c.c. horse serum intrav.	P +	+ T^2	+	T^2	+			T^2	+	T^2	+	T^2	+	T^2	+		T^2	+		T^2	+			T^2	+
66 Wt. 1520 grms.	5 c.c. horse serum intrav.	P +	+ T^2	+	T^2	+			T^2	+	T^2	+	T^2	+	T^2	+		T^2	+		T^2	+			T^2	+
3 Wt. 1600 grms.	5 c.c. horse serum intrav.	P −	+ T^2	±	T^2	+			T^2	−	T^2	−	T^2	−	T^2	+		T^2	+		T^2	+			T^2	+
9 Wt. 1670 grms.	5 c.c. horse serum intrav.	P +	+ T^2	+	T^2	±			T^2	+	T^2	+	T^2	+	T^2	+		T^2	+		T^2	+			T^2	+
48[1]		P +	+ T^1	+	T^2	+			T^2	+	T^2	+	T^2	+	T^2	+		T^2	+		T^2	+				s
2[2]							T^2	+						P T^2	+ +						T^2	+				

[1]Control in first serum reacting well.
[2]Jan. 7, 1 c.c. horse serum subcutaneously.
T =.01 c.c. horse serum in ear.
T^1 =.01 c.c. horse serum in abdomen.
T^2 =.1 c.c. horse serum in abdomen.
T^3 =.5 c.c. horse serum in abdomen.
P =Precipitine test.
s =skin reaction: one drop on shaved surface—scarified.

TABLE III.

Rabbit	No.	Jan. 19	20	21	22	23	24	25	26	27	28	29	30	31	Feb. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	70							T	−	T	−	T	−	T	−	T	−	T	+		T	+		T	+			T	+	
		0						P						P							P	+						P		
2	76													P	−						T	−						T	−	
														P	−						P	−						P		
3	54	0.1 c.c.						T	−	T	−	T	+	T	+	T	+	T	+		T	+		T	+			T	+	
								P	−					P							P	+						P		
4	52	serum												T	−						T	−						T	+	
														P	−						P	+						P		
5	22	0.5 c.c.						T	−	T	−	T	+	T	+	T	+	T	+		T	+		T	+			T	+	
								P	−					P	+						P	+						P		
6	79	serum												T	+						T	+						T	+	
														P	+						P	+						P		
7	82	1.0 c.c.						T	∓	T	∓	T	+	T	+	T	+	T	+		T	+		T	+			T	+	
								P	∓					P	+						P	+						P		
8	17	serum												T	±						T	±						T	+	
														P	+						P	+						P		
9	74	2.5 c.c.						T	+	T	+	T	+	T	+	T	+	T	+		T	+		T	+			T	+	
								P	+					P	+						P	+						P		
10	19	serum												T	−						T	±						T	+	
														P	+						P	+						P		
11	68	5.0 c.c.						T	+	T	−	T	+	T	−	T	+	T	+		T	+		T	+			T	+	
								P	+					P	+						P	+						P		
12	75	serum												T	+						T	+						T	+	
														P	+						P	+						P		
13	59													1.0 c.c.	−						T^1	−						T_1	−	T^1
																					P^1	−						P		

Rabbits 3 through 12 were injected intravenously on Jan. 19 with normal horse serum as indicated above.
Rabbit 13 was injected intravenously with 1.0 c.c. normal rabbit serum on Jan. 31.
T =1/100 c.c. normal horse serum injected subcutaneously.
T^1 =1/100 c.c. normal rabbit serum injected subcutaneously.
P =precipitine test: rabbit serum against normal horse serum.
P^1 =precipitine test: rabbit serum against normal rabbit serum.

each of these groups was injected with 0.01 cubic centimeter of horse serum intradermally. The rabbits in Groups 5 and 6, which had received the largest amounts of serum intravenously, showed definite local reaction on the seventh day. The susceptibility in these animals remained fairly constant after all subsequent injections.

On the twelfth day, all the animals were injected with 0.01 of 1 cubic centimeter of horse serum intradermally. Those receiving the test for the first time reacted nearly as readily as the other member of their group which had been tested several times before. This series, therefore, confirms the observation of Series 1 and 2 and adds the additional fact that a very small amount of horse serum is sufficient to produce a local skin reaction in a susceptible animal. A thirteenth rabbit, No. 59, was injected with 1 cubic centimeter of rabbit serum and tested on the seventh and fourteenth days with an intradermal injection of 0.01 cubic centimeter of rabbit serum with negative results.

Phenomena.—The local changes at or near the point of inoculation described as a reaction varied considerably at different times and in different animals. A typical reaction to horse serum in a sensitive rabbit may be described as follows: the bleb produced by the intradermal injection of the serum disappears in a short time, and no change is noticed at the seat of injection for ten to twelve hours. After this time the skin may become a little reddened and infiltrated, so that when it is taken up between the fingers in a fold, it seems definitely thickened as compared with a fold of skin elsewhere. The swelling and redness continue to increase and reach their height in about twenty-four hours, when there may be a definitely raised, brawny mass, perhaps one or two centimeters in diameter. The skin of the reacting area is red and increased in temperature, and the mass is definitely tender. Reactions which were less severe exhibited only a more or less diffuse infiltration of the skin at the point of inoculation with little or no redness or heat in the shaved area compared to that of the rest of the skin. In a number of rabbits the reaction was much more marked. In these the infiltration began sooner, the part affected extending five to ten centimeters, became very tense, swollen and red, and on several occasions partly necrotic. Reactions of this fulminating character often lasted for two or three weeks, whereas those which produced less severe changes diminished after twenty-four hours. On several occasions the reactions took the form of an unusually firm, sharply circumscribed round mass with less increase in local temperature and redness than was usually the case. It was thought

at one time that these firm nodules were the result of a skin test following closely the injection of a large amount (ten cubic centimeters) of horse serum intraperitoneally. We were unable, however, to reproduce later this form of reaction, and do not know to what factors it was due. As far as we could determine there was little constitutional disturbance during the time of reaction. The rabbits seemed lively and ate well. On several occasions following the injection of large amounts of serum intraperitoneally the rabbit appeared shocked. One had a short convulsive seizure followed by the passage of bloody urine. All these quickly and completely recovered. In the whole number of more than forty rabbits injected, no fatalities occurred.

Microscopic Appearances.—A typical swollen reacting area was excised for microscopic examination and may be described as follows:

The epidermis is everywhere intact and presents no abnormalities. Immediately beneath the epidermis the tissues were slightly infiltrated, but there is little if any accumulation of small round cells. Throughout the rest of the section of the skin and extending to the muscle layer, the tissues are definitely edematous, the fibrous strands being somewhat separated and compressed. In this area there is a marked increase in the number of leucocytes, many of them scattered, but the greater number gathered in small interstitial groups about the blood vessels. These cells about the vessels consisted mainly of polymorphonuclear leucocytes. The vessels are everywhere moderately injected and filled with red blood corpuscles. There is little if any evidence of marked hemorrhage, although some extravasation of red cells is noted about the smaller vessels. The muscle layer beneath the skin is unchanged. The whole appearance of this section suggested a moderately acute infiltration and inflammation.

Precipitine Test.—The serum of the rabbits was tested at intervals with horse serum for the presence of precipitines. The microscopic method was used for the most part, the results being controlled several times by microscopic test. The method used had been employed formerly by Dr. von Pirquet in agglutination of streptococci (4), and is briefly as follows:

On a cover slip three minute drops of horse serum, one undiluted, one diluted 1 to 10, and the third, 1 to 100, are placed in a row with a sterile platinum loop. Immediately opposite each drop of the horse serum is placed a drop of the same size of rabbit serum and the opposing sera are mixed gently together. The cover slip is now inverted over a hollow slide and placed in the incubator for three hours or for a longer time in room temperature, when under the microscope the precipitate is examined. When the precipitate forms, it is observed as a more or less extensive granular cloud. The number of times the precipitine test was made is indicated in Tables I, II, III. It will be noted that the precipitines were present in the blood of nearly all animals exhibiting local reaction, and were occasionally present where local reaction was not obtained. The dilution of the horse serum made but little difference in the intensity of the reaction.

Anti-anaphylaxis.—Having shown that the anaphylaxis, or the heightened susceptibility to a foreign serum could be demonstrated by a skin reaction, it was interesting to determine whether a lowered state of susceptibility, or the so-called anti-anaphylaxis, could be proved in the same way. Nicolle (3) first showed that experimental animals injected during incubation time not only were not killed by a second injection, but that their hyper-susceptibility was definitely lessened during this period. Besredka (5) and Rosenau and Anderson (6) working with guinea pigs found that when these animals survived a test injection they did not show general phenomena of inoculation again in twenty-four hours. In tuberculosis, also, it has been proven that after large doses of tuberculin subcutaneously, the reaction of the skin to vaccination with tuberculin (the von Pirquet test) disappears. A kind of tuberculin immunity develops. In our experiments we were unable to decrease the susceptibility of our rabbits to the intradermal injections of horse serum. Repeatedly sensitized animals which were exhibiting definite local reactions to every injection of horse serum were given large doses, five or ten cubic centimeters, intravenously or intraperitoneally, but an intradermal injection made within twenty-four hours always produced a definite local reaction. In several instances after a procedure of this kind, the localized swelling was

usually hard and circumscribed, but this form of reaction could not be regularly obtained.

Transmission of Susceptibility to Offspring.—A number of experiments were carried on to determine whether it was possible to transmit this susceptibility, which is developed in rabbits, and indicated by the local skin reaction, to their young. Anderson (7) had shown that the toxic hyper-susceptibility to horse serum, as indicated by the general phenomena of anaphylaxis and ending in death, can be transmitted in guinea pigs from a sensitized mother to her young. In several experiments the injection of horse serum into the offspring of such sensitized mothers was fatal in less than an hour, while much larger quantities of horse serum produced no effect upon young guinea pigs from unsensitized mothers. Two litters of rabbits, one six in number and three weeks old, from the offspring of a highly sensitized mother which had markedly reacted just before the birth of her young, were inoculated with 0.02 cubic centimeters of horse serum every other day intradermally into the abdomen, and on the fifth day following developed a definite but slight thickening and infiltration of the skin a few hours after the injection. At the same time a litter of five rabbits from an unsensitized mother were similarly injected and reacted positively in just the same period of time, but more vigorously than did those of the first litter. The reactions following the last injections in the second series were all accompanied by marked induration and considerable hemorrhage. Our results suggest, therefore, that the susceptibility of the sensitized rabbit, as indicated by the skin reaction, is not freely transmitted to its offspring. A comparison of the effects upon the young of these two litters would suggest, however, the possibility that the offspring of the sensitized rabbit have acquired some allergy to horse serum, that is, the property of an accelerated formation of ergines (8).

Leucocytic Count.—It was thought that it would be of interest to determine whether the leucocytes underwent any change either in their total number or differential count following the sensitizing injection of serum or following the skin reaction induced by the subsequent intradermal injection of minimal doses of serum. To determine these points four fresh rabbits of approximately the same

age and weight were selected, and before any injections were given, leucocyte counts were made as follows: at nine a. m., one p. m., and five p. m., on the first day, and at nine a. m. on each of the three successive days. After a rest of about a week, a similar series of counts were made. Another week was allowed to elapse and then sensitizing injections of serum were made intravenously. The first rabbit was given 0.1 cubic centimeter, the second, 2.5 cubic centimeters, and the third, 5 cubic centimeters, while the fourth was not injected and served as a control. The leucocytes were then counted exactly as on the two preceding occasions. After a lapse of fourteen days all four rabbits received intradermal injections of 0.01 cubic centimeters horse serum and a fourth series of counts was carried out. The total number of leucocytes and differential counts were made on each occasion, in all twenty-four total leucocytes and twenty-four differential counts on each rabbit.

The leucocytes varied through wide limits both in their total number and in the differential counts, the variations being just as great in the two series of counts made before any serum had been injected as in those series following the sensitizing injection and the subsequent test injection, and no relation could be made out between the injections and the leucocyte curves.

The detailed results of these counts are too long to include here. They will be reported in a separate paper.

CONCLUSIONS.

1. Anaphylaxis or allergy of rabbits against horse serum can be proved by subcutaneous test.

2. The test is best made in the following way. The skin of the animal, preferably of the abdomen or flank, is shaved. (This should be done a few hours before the injection.) The injection is made by means of a small hypodermic syringe and intradermally. An effort was made not to inject the serum under the skin. Those injections were considered most favorable by which the serum remained as a small bleb in the skin proper. Undiluted horse serum was used for most of the experiments. The amount injected varied from 0.01 cubic centimeter to 1 cubic centimeter. The reaction seemed as definite after 0.01 cubic centimeter as after a larger quantity.

Rabbit.	No.	Intravenous Injection.	Nov 25	26 1	27 2	28 3	29 4	30 5	Dec. 1 6	2 7	3 8	4 9	5 10	6 11	7 12	8 13	9 14	10 15	11 16	12 17	13 18	14 19	15 20	16 21	17 22	18 23	19 24	20 25	21 26	22 27	23 28	23 28	24 29	25 30	26 31	27 32	28 33
1	48	0							T	−	T	±	T	±	T	−	T^1	+	T^2	+			T^2	+						I^1		P	+					
2	37															P	−	T^1	−											T^2	−	P	−					
3	55	0.1 c.c. serum							T	−	T	−	T	+	T	−	T^1	+	T^2	+			T^2	+						T^2	+							
4	55															P	−	T^1	±											T^2	±							
5	51	0.1 c.c. serum +9.9 c.c. normal salt solution							T	−	T	−	T	+	T^1	+	T^1	±	T^2	±			T^2	+						T^2	−							
6	27															P	+	T^1	−											T^2	−	P	−					
7	26	0.5 c.c. serum							T	−	T	−	T	±	T^1	±	T^1	+	T^2	+			T^2	+						T^2	−							
8	78															P	−	T^1	±											T^2	+	P	+					
9	42	0.5 c.c. serum +9.5 c.c. normal salt solution							T	−	T	−	T	+	T^1	+	T^1	−	T^2	+			T^2	±						T^2	+							
10	71															P		T^1	−											T^2	−	P	±					
11	56	1.0 c.c. serum							T	−	T	−	T	±	T	−	T^1	+	T^2	+			T^2	+						T^2	−							
12	30															P	+	T^1	−											T^2	−	P	+					
13	4	1.0 c.c. serum +9.0 c.c. normal salt solution							T	−	T	−	T	±	T		±	T^1	±	T^2	+			T^2	+						T^2	+							
14	49															P	+	T^1	+											T^2	+	P	+					
15	14	5.0 c.c. serum							T	−	T	−	T	+	T^1	+	T^1	±	T^2	+			T^2	+						T^2	+							
16	35															P		T^1	±											T^2	+	P						
17	34	5.0 c.c. serum +5.0 c.c. normal salt solution							T	−	T	−	T	+	T^1	+	T^1	−	T^2	+			T^2	±						T^2	+							
18	40															P	+	T^1	+											T^2	+	P	+					
19	50	10.0c.c. serum							T	−	T	±	T	+	T	−	T^1	+	T^2	+			T^2	+						T^2	+							
20	10															P	+	T^1	+											T^2	−	P	+					

T =inoculation of 0.1 c.c. 10 per cent. serum subcutaneously in ear.
T^1 =inoculation of 0.1 c.c. 10 per cent. serum subcutaneously in abdomen.
T^2 =inoculation of 0.1 c.c. undiluted serum subcutaneously in abdomen.
T^3 =inoculation of 0.05 c.c. undiluted serum subcutaneously in abdomen.
T^4 =0.01 c.c. horse serum.
P =precipitine test.

TABLE I.

23 28	23 28	24 29	25 30	26 31	27 32	28 33	29 34	30 35	31 36	Jan. 1 37	2 38	3 39	4 40	5 41	6 42	7 43	8 44	9 45	10 46	11 47	12 48	13 49	13 49	14 50	15 51	16 52	17 53	18 54	19 55	20 56	20 56	21 57	22 58	23 59	24 60	25 61	26 62	27 63	28 64	29 65	30 66
	P +																											T^3	+		T^2	+	T^4	+	T^2	+	T^4	+	T^2	+
−	P −							T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
+								T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
±								T^2	−				T^2	−								P	+	T^2	±				P	−	T^3	−					T^2	−		
−								T^2	−				T^2	−								P	+	T^2	+				P	+	T^3	+					T^2	+		
−	P −							T^2	−				T^2	−								P	±	T^2	±				lost P	−	T^3	±					T^2	±		
−								T^2	+				T^2	+								P	+	T^2	+				P	−	T^3	−					T^2	+		
+	P +							T^2	+				T^2	−								P	+	T^2	±				P	+	T^3	+					T^2	−		
+								T^2	+				T^2	±								P	+	T^2	+				P	+	T^3	+					T^2	+		
−	P ±							T^2	−				T^2	−								P	±	T^2	−				P	±	T^3	−					T^2	−		
−								T^2	+				T^2	−								P	+	T^2	+				P	+	T^3	+					T^2	+		
−	P +							T^2	−				T^2	−								P	+	T^2	+				P	+	T^3	+					T^2	+		
+								T^2	+				T^2	+								P	+	T^2	+				P	±	T^3	+					T^2	+		
+	P +							T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
+								T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
+	P							T^2	+				T^2	−								P	+	T^2	+				P	+	T^3	−					T^2	+		
+								T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
+	P +							T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
+								T^2	+				T^2	+								P	+	T^2	+				P	+	T^3	+					T^2	+		
−	P +							T^2	+				T^2	+								P	±	T^2	+				P	+	T^3	+					T^2	+		

I = 10 c.c. horse serum intraperitoneally.
I^1 = 10 c.c. horse serum (half went into periton
I^2 = 10 c.c. horse serum intravenously.

	Feb.																						
31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
T^2	+	T^2	P +	T^2	+		T^2	+		T^2	+				T^4	$-T^4$	−		I^2T^2	+			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	−												
			T^2	+																			
			P	+						T^2	−												
			T^2	+																			
			P	+						T^2	−												
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	−												
			T^2	−																			
			P	+						T^2	+						IT^2	−	T^2	+			
			T^2	−																			
			P	+						T^2	+								I^2T^2	+			
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	+					I	T^2	+					
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	+								IT^2	+			
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	+												
			T^2	+																			
			P	+						T^2	+								IT^2	+			
			T^2	+																			
			P	+						T^2	+								IT^2	−			
			T^2	+																			

um and half into subcutaneous tissue).

3. The specific reaction appears in from twelve to twenty-four hours after the test is made and reaches its maximum in from twenty-four to thirty-six hours. It consists of a local swelling extending from 0.5 to 2 centimeters from the point of inoculation. The skin involved in the raised area is usually red and hotter than the surrounding skin. Macroscopically and microscopically the reacting area has the appearance of a local acute inflammation.

4. The altered reactivity (allergy) or hyper-susceptibility (anaphylaxis) sets in usually in from ten to fifteen days after the first injection of horse serum, and lasts at least three months. Individual rabbits show marked variation from the average time of the development of anaphylaxis.

5. The appearance of precipitines against horse serum in the blood of rabbits appears nearly synchronously with the allergic condition.

6. After large injections of serum the allergic rabbits still react subcutaneously. A suppression of allergy which would correspond to the so-called anti-anaphylaxis could not be proved.

7. Also in regard to the offspring of injected rabbits the subcutaneous test was not positive. The young of these rabbits did not develop a more active allergy than the young of normal rabbits.

8. Neither the injection of considerable quantities of horse serum nor the development of a marked local reaction in the skin after intradermal inoculations of horse serum in a sensitized rabbit is accompanied or followed by greater variations in the number or types of leucocytes in the circulating blood than is found in control animals.

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